
# Comparative studies on *Macrocystis pyrifera* and *Ecklonia maxima* (Laminariales) in South Africa; investigating the use of *M. pyrifera* as abalone feed in South African aquaculture and assessing the vulnerability of these two species to Marine Heat Waves

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**Thesis presented for the degree of Master of Science**

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**February 2019**

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29/01/2019

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Date

**Acknowledgements**

I would like to thank the following people:

John Bolton and Mark Rothman for the supervision, support and wisdom they have provided me throughout my postgraduate studies. Chris Boothroyd and Derek Kemp from the Department of Environment, Forestry and Fisheries, Seaweed Research Unit, for their invaluable assistance and support they have provided throughout my Masters. Maggie Reddy and David Dyer for their assistance with project work and keeping me company in the phycology lab.

Nick Loubser of Viking Fisheries Aquaculture for facilitating the commercial abalone feed inclusion trial through the provision of site, animals, equipment and feeds. Pieter and Cara Marais for their continued assistance throughout the trial period.

The University of Cape Town, Biological Sciences Department, Dawood Hattas and Bongani Tom for providing access to laboratory facilities and making available any equipment I required.

My wonderful parents, Jane and David Fleischman for the love, support and wisdom they have provided to me all my life, without which I wouldn't be the person I am today. My brothers Nicholas, Matthew, Sebastian and Milo Fleischman, for showing interest in my work and their brotherly love. Lastly my girlfriend, Danielle Goosen, for her assistance with figure development, patience, understanding, constant encouragement, advice and uncontainable love.

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**Abstract**

*Macrocystis pyrifera* is a kelp species found along many rocky temperate coastlines throughout the world. This species has been the focus of much research in recent decades, owing to its important ecological role, as well as its many commercial uses. Along the South African coastline *M. pyrifera* exists in eight known populations, over a limited *ca.* 200 km distribution. South African *M. pyrifera* differs from other global populations, as it is shorter in length and develops as small populations, often inshore of populations of the widely distributed and commercially valuable *Ecklonia maxima*. Very little research has been carried out on South African *M. pyrifera*. This thesis comprises two comparative studies on South African *M. pyrifera*, carried out with the aim of increasing our knowledge about the species in South Africa.

South African abalone aquaculture has been increasing for many years and farmers are continually searching for new sustainable sources of abalone feed. As more abalone farms are built and existing farms expand, farms that use kelp as a feed, will likely require increased fresh kelp biomass in the future. The first study investigated *M. pyrifera* as a potential alternative source of supplementary fresh kelp feed for the South African abalone aquaculture industry. A six month comparative abalone feed inclusion trial and growth study was carried out, where the commercially farmed abalone species, *Haliotis midae*, was provided a diet of either *E. maxima* (the primary fresh kelp diet in the industry) + SAF 3000® formulated feed or *M. pyrifera* + SAF 3000® formulated feed. *Haliotis midae* fed with *M. pyrifera* + SAF 3000® displayed comparable growth rates to abalone fed a diet of *E. maxima* + SAF 3000®. No significant differences in the resultant mean Specific Growth Rate (*M. pyrifera*- 0.19 ± 0.01, *E. maxima*- 0.18 ± 0.01 % body weight.day$^{-1}$; $F= 0.180$, $df_1= 1$, $df_2= 192$, $p=$

0.672), Daily Increment in Shell Length (*M. pyrifera*- 31.19 ± 2.59, *E. maxima*- 24.64 ± 2.31 $\mu m.day^{-1}$; $F= 0.853$, $df_1= 1$, $df_2= 192$, $p= 0.357$) and mortality rate ($t(10)= 0.263$, $p= 0.789$) were identified between the two diets provided. Mean abalone Condition Factor also showed no significant differences among diets (*M. pyrifera*- 1.37 ± 0.01, *E. maxima*- 1.40 ± 0.01 $g.mm^{-1}$), with the exception of month 2 ($F= 4.014$, $df_1= 1$, $df_2= 224$, $p< 0.05$). Additionally, *H. midae* consumed significantly less *M. pyrifera* (31.76 ± 2.83 %) than *E. maxima* (72.70 ± 1.26 %) ($t(18)= 13.218$, $p< 0.05$), suggesting potential differences in the Feed Conversion Ratio and/or kelp nutritional composition amongst these two kelp species. *Macrocystis pyrifera* could thus serve as a possible alternative supplementary kelp feed for the South African abalone industry. However, a sustainable source of *M. pyrifera* would be required. Moreover, further studies that examine the Feed Conversion Ratio, nutritional content and other important parameters relative to the use of *M. pyrifera* in aquaculture, should be established to reinforce these findings.

At present, anthropogenic climate change arguably poses the largest threat to the world's oceans. Rising ocean temperatures will increase climatic variability, leading to increases in the frequency, duration and intensity of extreme climatic events such as storms and Marine Heat Waves (MHW's). The second study investigated the impact of MHW's on both *M. pyrifera* and *E. maxima* recruits. Recruits of both species were exposed to an experimentally simulated MHW for a 72 hour period. Recruits were subjected to four temperature treatments: 15, 17.5, 20 and 22.5 °C. The impact of the simulated MHW on the samples was measured via photosynthetic oxygen production. Evidence of thallus deterioration in the samples was also recorded. The findings demonstrated that the thermal tolerance ranges and optima varied among the two species, with *M. pyrifera* displaying a greater sensitivity to the simulated MHW. Both

species indicated minimal thallus deterioration at 15 °C and 17.5 °C. However, at 20 °C, 80 % of the *M. pyrifera* recruits and 40 % of the *E. maxima* recruits displayed signs of thallus deterioration. This increased to 100 % of *M. pyrifera* recruits and 80 % *E. maxima* recruits in the 22.5 °C treatment, exhibiting thallus deterioration. Mean oxygen production by *M. pyrifera* was greatest at 15 °C (1.42 ± 0.12 mg $O_2.g^{-1}$ $DW.h^{-1}$), with oxygen production significantly declining above 17.5 °C ($F= 18.410$, $df_1= 3$, $df_2= 16$, $p< 0.05$). Therefore, temperatures just above 17.5 °C likely lie at the upper end of the thermal tolerance range of South African *M. pyrifera*, with a thermal optima potentially existing at a lower temperature than what was tested. Mean oxygen production by *E. maxima* was greatest at 17.5 °C (1.92 ± 0.19 mg $O_2.g^{-1}$ $DW.h^{-1}$) and was significantly reduced at 22.5 °C ($F= 4.987$, $df_1= 3$, $df_2= 16$, $p< 0.05$). *Ecklonia maxima* thus possesses a larger thermal tolerance range in comparison with *M. pyrifera*, with temperatures between 20 and 22.5 °C representing the upper limit of thermal tolerance for this species. The findings of this study highlight the threat of MHW's to South African *M. pyrifera* and *E. maxima*. Furthermore, the findings assist in explaining the limited distribution of *M. pyrifera* along the South African coastline. However, further research is required to fully understand the implications of MHW and other warming events on the persistence of these two species in a climate change future.

Chapter 1-

General introduction

A low number of contributions are available on the South African populations of *Macrocystis pyrifera* (Linnaeus) C. Agardh, elsewhere known as 'giant kelp'. This thesis aims to document and extend available knowledge about this species and its South African populations. Here I present two comparative studies on the South African kelp species, *M. pyrifera* and *Ecklonia maxima* (Osbeck) Papenfuss (the dominant kelp species making up South African kelp forests). The first study assesses the viability of *M. pyrifera* as an alternative source of supplementary kelp feed for the South African abalone aquaculture industry. This was done through a comparative feed inclusion trial where the abalone species, *Haliotis midae* Linnaeus, was grown on diets composed of either *M. pyrifera* or *E. maxima* (both diets also included SAF 3000® formulated feed). The second study examines the effect of Marine Heat Waves (MHW's) on *M. pyrifera* and *E. maxima*. Both species were subjected to an experimentally simulated MHW for a 72 hour period, after which the impact of the MHW on productive capacity was measured via oxygen production.

Kelp, basic kelp taxonomy and global distribution

Kelp are highly productive and complex organisms, which form the foundation of the kelp forest ecosystem (Steneck et al., 2002). Kelp forest ecosystems display high levels of primary productivity and organism diversity comparable to terrestrial tropical rain forests (Filbee-Dexter et al., 2016). The importance of kelp to both humans and coastal temperate ecosystems has resulted in kelp and kelp communities being the focus of much research (Teagle et al., 2017; Wernberg et al., 2019).

The word ‘kelp’ originated as a term used to describe the calcined ashes formed by the burning of large brown algae. Nowadays, kelp generally refers to the species of large brown algae within the order Laminariales (Schiel & Foster, 2015). The high level taxonomic classification of these organisms is still largely debated and revisions of species classification within this order is common. However, the Cavalier-Smith (2010) classification scheme is currently the most widely accepted classification among phycologists. In this classification, kelp are placed within the kingdom Chromista, phylum Ochrophyta, class Phaeophyceae and order Laminariales (Cavalier-Smith, 2010; Guiry & Guiry, 2019).

Kelp dominate rocky reefs in lower intertidal and shallow subtidal habitats along much of the world’s temperate, sub-polar and arctic coastlines (Dayton, 1985; Steneck et al., 2002; Teagle et al., 2017) (Fig. 1). Within their global distributions, kelp species commonly grow in high densities, forming what are known as kelp forests. Global kelp forest distributions are determined and influenced by a wealth of environmental factors including substrata type, water motion/wave action, sedimentation, light availability, salinity, ocean temperature and nutrient availability (Dayton, 1985; Shukla & Edwards, 2017). At high latitudes, the biogeographical limit of kelp distribution is largely constrained by light, nutrient availability, low temperatures, ice scour and evolutionary biology (as a result, kelp extend further into higher latitudes in the northern hemisphere) (Dunton & Dayton, 1995), while at low latitudes, kelp are limited by high ocean temperatures and nutrient availability (Steneck et al., 2002). Additionally, biotic factors such as spore dispersal, successful recruitment, spore survivorship and physiological adaptations to dispersal also play a role in determining the distribution of kelp species (Steneck et al., 2002).

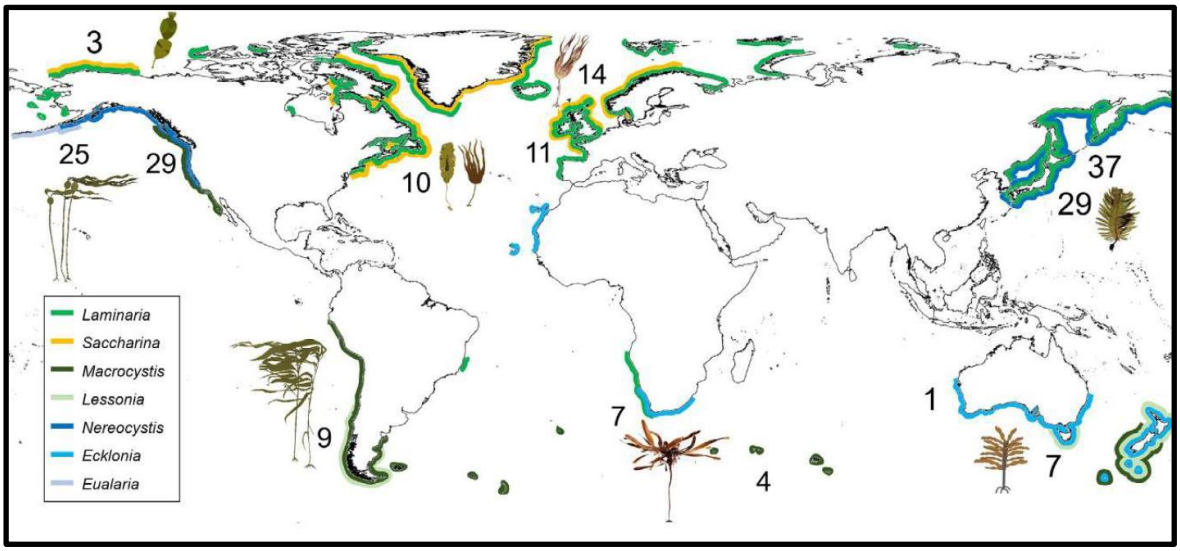


**Figure 1.** Map showing the predominantly anti-tropical global distribution of Laminarian kelp, with dominant genera indicated (coloured lines) and number of species (Wernberg et al., 2019).

Kelp phenology, sporophyte structure and ecological role

Within their global distributions, kelp are often subjected to strong seasonal differences in the physical environment, where annual fluxes in temperature, light and nutrient availability influences kelp phenology (Schiel & Foster, 2015). A consequence of this are seasonal variations in kelp growth and reproduction. For example, most kelp display maximum growth in late winter and early spring, when ocean temperatures are low and nutrient availability is high. This is followed by increased photosynthesis and photosynthetic product storage in summer, when light intensity is greater (Graham et al., 2007; Krumhansl & Scheibling, 2011; Wernberg et al., 2019).

All kelp share a common life cycle, consisting of an alternation of generations between a microscopic haploid gametophyte stage and large macroscopic diploid sporophyte stage (Dean & Jacobsen, 1984; Dayton, 1985; Schiel & Foster, 2015) (Fig. 2).

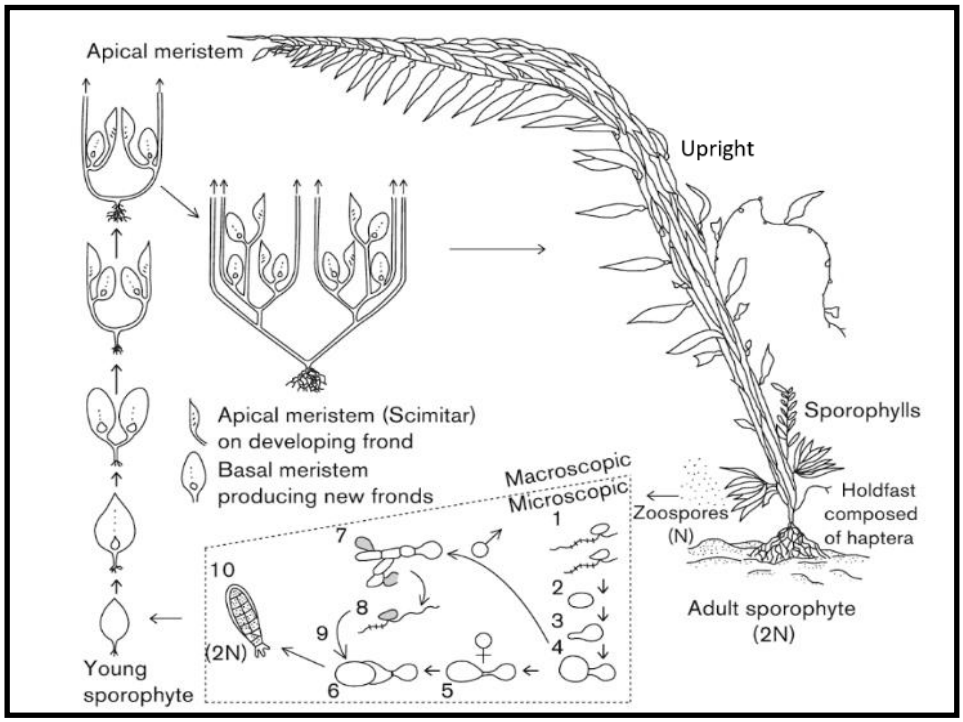


**Figure 2.** Diagram showing the life cycle of *Macrocystis pyrifera*, from zoospore to adult sporophyte (Schiel & Foster, 2015).

Kelp species either reproduce seasonally or will continually reproduce throughout the year. Furthermore, the timing of reproduction can vary among geographically separate kelp populations of the same species, due to differing environmental conditions (Dayton, 1985; Graham et al., 2007; Mohring et al., 2013; Schiel & Foster, 2015). For example, *M. pyrifera* populations in southern Chile have been shown to exhibit year-round reproduction, while northern Chilean populations display a spring-winter seasonal reproductive cycle, with wave action believed to be driving reproductive differences (Buschmann et al., 2004). Additionally, Joska & Bolton (1987) found South African *E. maxima* to display year-round fertility, however, fertility levels varied temporally, peaking in spring. Therefore, both seasonal cycles and local environmental conditions may influence kelp development and reproduction.

Strong differences in kelp morphology also exist, both among and within a species (Steneck et al., 2002). For example, *Ecklonia radiata* (C. Agardh) J. Agardh in Australia and South Africa has been shown to display polymorphism, with distinct differences in stipe length, spinosity, rugosity, frond thickness and frond density among populations (Rothman et al., 2015). Despite these differences, kelp tend to share a similar underlying structure. Most kelp are attached to the substratum by branched, intertwined terete haptera known as a holdfast. From the holdfast, extends one or more stipes upwards, elevating the kelp to the sea surface where there is more light. A few species of kelp also possess gas-filled floats, which aid in maintaining the kelp's position in the water column. At the upper end of the stipe, a single large or multiple smaller blades (lamina) exist, which carry out photosynthesis and create a large surface area for gas and nutrient transfer (Stekoll & Else, 1990; Wernberg et al., 2019).

Kelp and kelp forests are invaluable to the environments in which they occur. Their complex three dimensional structure and high productivity allows them to be a source of habitat and food to a wealth of associated organisms (Field et al., 1980a; Steneck et al., 2002; Filbee-Dexter et al., 2016; Teagle et al., 2017; Blamey & Bolton, 2018). Organisms associated with kelp forests include molluscs, teleosts, marine mammals, echinoderms, crustaceans and many other associated algal species (Steneck et al., 2002). Kelp are also important primary producers and are highly influential ecosystem engineers along temperate coastlines (Biskup et al., 2014; Teagle et al., 2017; Blamey & Bolton, 2018). Their presence alters ecological factors such as light availability, substratum availability, sedimentation influx, flow dynamics, benthic productivity, organism recruitment, competition and the quantity of food (Teagle et al., 2017; Filbee-Dexter & Wernberg, 2018). Additionally, the biogenic habitats kelp create,

provide shelter to key ecological species, serve as nursery grounds for juvenile fish and enhance community diversity. Kelp forests also carry out nutrient recycling and offer biogenic coastal defence, providing resilience to other nearby marine and terrestrial ecosystems (Bolton & Anderson, 1997; Steneck et al., 2002; Teagle et al., 2017). Therefore, kelp have a large influence on both near-shore ecological and oceanographic processes, making them important components of temperate coastlines (Steneck et al., 2002).

The importance of kelp to humans and global kelp utilisation

Kelp have also been of value to humans for tens of thousands of years. Humans first exploited the resources produced by kelp forests around 10,000 - 70,000 years ago (Volman, 1978). It is suggested that early humans, who lived along the Southern African coastline, were supported by rich diets comprising of associated kelp forest organisms (Erlandson et al., 2007; Blamey & Bolton, 2018).

Currently, kelp forest ecosystems provide many social, economic and ecological ecosystem services including climate control, carbon sequestration, coastline protection, nutrient filtering, facilitate tourism and support commercially and recreationally important fisheries (DeMartini & Roberts, 1990; Duarte et al., 2013; Blamey & Bolton, 2018; Filbee-Dexter & Wernberg, 2018). As a result, the services provided by kelp forests are suggested to be worth billions of dollars annually, with an estimated value of US$ 500,000 - 1,000,000 $km^{-1}$ of kelp ecosystem coastline (Teagle et al., 2017; Blamey & Bolton, 2018; Filbee-Dexter & Wernberg, 2018). The use of kelp by humans has increased globally over the last decade, doubling to over 8 million tons in 2014, due to a rising demand for kelp-derived products for use in industrial processes and as human food (particularly 'Kombu'-*Saccharina japonica* (Areschoug)

C.E. Lane, C. Mayes, Druehl & G.W. Saunders, and 'Wakame'- *Undaria pinnatifida* (Harvey) Suringar, which are largely produced via aquaculture and used as everyday foods in Japan and China) (Chapman & Chapman, 1980; Fleurence et al., 2012; Buschmann et al., 2014; Borras-Chavez et al., 2016, Sanjeewa & Jeon, 2018; Wernberg et al., 2019). Kelp and their chemical constituents have a variety of applications including the human food industry, animal fodder, biofuel, the chemical industry (pharmaceuticals, nutraceuticals and cosmetics) and as a source of alginic acid ('alginate'- a complex polysaccharide compound found in the cell walls of kelp, with uses in a range of markets) (Buschmann et al., 2014, Schiel & Foster, 2015; Borras-Chavez et al., 2016; Camus et al., 2018; Wernberg et al., 2019).

Additionally, kelp are harvested from natural populations for their use as a feed in abalone aquaculture (Borras-Chavez et al., 2016). Abalone were first cultured in land-based aquaculture systems in the 1960's in Japan and the USA. The industry has since expanded globally, with abalone aquaculture now occurring in Australia, New Zealand, Chile, USA, Mexico, China, Taiwan Korea and South Africa (Flores-Aquilar et al., 2007; Hernández et al., 2009; Cook, 2014). While not all land-based abalone aquaculture facilities use kelp as a feed, those that do require high levels of kelp biomass to grow abalone to marketable size (Hernández et al., 2009).

Natural and anthropogenic threats to global kelp populations

At mid-latitudes, herbivory by sea urchins forms an important means of kelp forest turnover via deforestation (Foster, 1990). However, the formation of phenomena known as 'urchin fronts' and subsequent 'urchin barrens' have the potential to severely impact kelp forest ecosystems and disrupt normal functioning (Dean et al., 1984; Johnson et al., 2011; Filbee-Dexter & Scheibling, 2014; Teagle et al., 2017). These

events occur when the activity of top down predation is slowed or stopped (i.e. due to overfishing and other human activities), resulting in rapid increases in local urchin populations, which aggregate into fronts (Steneck et al., 2002; Filbee-Dexter & Scheibling, 2014; Teagle et al., 2017). Urchin fronts feed on young kelp and adult kelp holdfasts, inhibiting recruitment and leading to holdfast structural failure in adults (Dean et al., 1984; Tegner et al., 1995). This creates what is known as an urchin barren - an environment devoid of fleshy and filamentous algae, which can vary from hundreds of meters to thousands of kilometres in spatial extent. These environmental states can occur abruptly and persist for long periods of time, due to continued urchin presence restricting kelp recruitment and creating a positive feedback mechanism (Dean et al., 1984; Tegner et al., 1995; Johnson, et al., 2011; Filbee-Dexter & Scheibling, 2014).

Storms play an important role as a restructuring agent in kelp forests, allowing for the recruitment of new individuals through the removal of older kelp (Santelices & Ojeda, 1984; Ebeling et al., 1985; Byrnes et al., 2011; Teagle et al., 2017). Wave action and storms can also assist in the dispersal of kelp and associated biota (Batista et al., 2018). However, frequent storms and wave induced kelp entanglement can lead to the breakage, dislodgement and removal of large sections of kelp forests. The loss of kelp often results in the loss of associated kelp forest organisms, reducing organism diversity (Ebeling et al., 1985; Seymour et al., 1989; Byrnes et al., 2011; Reed et al., 2011). For example, *M. pyrifera* along Southern California are regularly cleared by storms, sometimes as frequently as every one to three years. After the loss of these kelp forests it can take many years until normal ecological functioning returns (Ebeling et al., 1985; Seymour et al., 1989; Reed et al., 2011).

The El Niño Southern Oscillation (ENSO) is a climate phenomenon, resulting from the weakening of the Pacific westerly trade winds. ENSO events have the potential to slow and disrupt upwelling systems along the east Pacific and result in an influx of warm, nutrient poor surface waters into coastal areas (Tegner & Dayton, 1991; Dayton et al., 1999). This creates stressful conditions for east Pacific kelp populations, negatively influencing large-scale kelp distributions along the temperate coasts of North and South America in the past (Tegner & Dayton, 1991; Tegner et al., 1996; Gerard, 1997). For example, ENSO events in the early 1980's and late 1990's resulted in large scale canopy die-off and the loss of many *M. pyrifera* kelp populations along southern California. During these events, temperature anomalies of up to 3 – 4 °C warmer than the previous 10 year average were recorded (Tegner & Dayton, 1991; Tegner et al., 1996; Ladah et al., 1999; Hernández-Carmona et al., 2001).

Anthropogenic activities also pose direct and indirect threats to global kelp ecosystems. Decreasing water quality and eutrophication, due to human expansion, population growth and subsequent human pollution and the overfishing of apex predators can result in negative cascade effects, altering kelp forest community composition (Steneck et al., 2002; Johnson et al., 2011; Teagle et al., 2017; Wernberg et al., 2019). Furthermore, the over-harvesting of kelp for commercial use threatens the biodiversity and normal functioning of kelp forests and surrounding ecosystems (Christie et al., 1998; Anderson et al., 2006; Teagle et al., 2017).

Lastly, anthropogenic global climate change and the resultant ocean warming poses one of the largest threats to kelp forest ecosystems (Steneck et al., 2002; Wernberg et al., 2010). Kelp persist in temperate waters and become physiologically stressed by high ocean temperatures. High ocean temperatures also increase kelp susceptibility to

disease and creates pathogen friendly environments (Wernberg et al., 2010; Wahl et al., 2015). In addition to rising ocean temperatures, climate change models predict an increase in the frequency and intensity of storms and Marine Heat Waves (MHW's), due to ocean warming (Byrnes et al., 2011; Schlegel et al., 2017). MHW's are defined as "a prolonged discrete anomalously warm water event that can be described by its duration, intensity, rate of evolution, and spatial extent" (Hobday et al., 2016). MHW's are caused by atmosphere-ocean heat transfer or from advection (transport of warm water via currents) (Schlegel et al., 2017). Increases in storm activity and MHW's will have negative consequences for global kelp forest distributions, particularly those at the edge of the subtropical range where these events will be most intense (Teagle et al., 2017; Wernberg et al., 2019).

Much evidence for the negative impacts of rising ocean temperatures and MHW's on kelp populations already exists. For example, increases in ocean temperature in Nova Scotia, Canada (0.064 °C in summer and 0.039 °C per year) over the last three decades has resulted in many of highly productive local kelp populations being replaced by 'turfs', largely made up of invasive algae. Consequently, it is estimated that mean kelp biomass has decreased by between 85 - 99 % along the north-west Atlantic coastline (Filbee-Dexter et al., 2016). Moreover, ocean warming and the intensification of the East Australian Current has caused rapid declines in the *M. pyrifera* kelp forests along the Tasmanian coast since the 1980's, with large alterations to associated communities (Johnson et al., 2011). The resultant loss of kelp forests in these regions will see reductions in food web and community complexity and create niches for invasion by alien species. Therefore, anthropogenic climate change has the potential to impact the structure, productivity, distribution and resilience of global kelp forests and will drive

global reductions in the quantity and quality of kelp forest ecosystems (Steneck et al., 2002; Wernberg et al., 2010, 2011; Johnson et al., 2011; Teagle et al., 2017).

The South African coastal environment

The Southern African coastline is characterised by two main currents; the warm Agulhas Current, which flows down the east coast, and the cool Benguela Current along the west coast (Stegenga et al., 1997; Blamey et al., 2015). The west coast is classified as a cool temperate region, with monthly temperatures falling between cold and warm temperate conditions (Schlegel et al., 2017). Annual mean temperatures along the west and south-west coast range from 12-16 °C, depending on location. Temperatures increase south and eastward, making the south-west coast warmer on average than the west coast (Stegenga et al. 1997; Smit et al., 2017). Along the west coast, prevailing south-easterly winds interacting with the Benguela current, drive the Benguela upwelling system (Bolton & Levitt, 1987; Stegenga et al., 1997; Schlegel et al., 2017). This is one of four major eastern boundary upwelling systems that exist globally and is divided into a northern and southern system. The northern Benguela system is located off the southern Angolan and Namibian coasts, while the southern Benguela runs along the west and south-west coasts of South Africa, with its eastern boundary at Cape Agulhas (Shannon et al., 2003; Shillington et al., 2006; Blamey et al., 2015; Lamont et al., 2018).

A wealth of research has been carried out on this upwelling system, highlighting its high level of complexity and ecological importance. Upwelling along the southern Benguela occurs predominantly from austral spring to late summer, driving seasonal fluctuations in physical factors in the local marine environment (Velimirov et al., 1977; Bolton & Levitt, 1987; Hutchings et al., 2009; García-Reyes et al., 2018; Veitch et al.,

2018). During times of upwelling, nearshore waters can be exchanged at a high rate, up to seven times per day in some locations, creating dramatic variations in temporal physical conditions (Field et al., 1980b).

Most marine environmental factors, including light, nutrients and temperature, are influenced by upwelling. Upwelling brings nutrient rich, cool sub-surface waters to the surface (Shannon, 1985; Shillington et al., 2006), which is often clearer (due to low plankton abundance), increasing light availability in the water column (Bolton & Levitt, 1987). Inshore annual mean temperature variability along the south coast can be far greater than the west coast (up to 7 °C, in comparison with 2 and 4 °C in the coldest and warmest months in the Benguela region, respectively) (Shannon, 1989). However, upwelling events can rapidly cause temperatures (and associated nutrient levels) along the west coast, to fluctuate over the monthly mean maximum range within several days (Bolton & Levitt, 1987; Smit et al., 2017). Additionally, the west coast experiences a high level of wave action, with average prevailing south-westerly swells ranging from 2 - 3 m and reaching maximum heights of 5 - 7 m, thus creating a highly turbulent marine environment (Rothman et al., 2017). Therefore, the west and south-west coast of South Africa are characterised by highly variable marine conditions, with unique biota and physico-chemical features (Bolton & Levitt, 1987; Blamey et al., 2015).

*Ecklonia maxima* and *Macrocystis pyrifera* in South Africa

Four species of kelp grow in South Africa; *Ecklonia maxima*, *Ecklonia radiata*, *Laminaria pallida* Greville ex J. Agardh and *Macrocystis pyrifera* (Stegenga et al., 1997). *Laminaria pallida* and *M. pyrifera* both fall under the family Laminariaceae, while the two *Ecklonia* species belong to the family Lessoniaceae (Guiry & Guiry, 2019)

(although findings by Jackson et al. (2017) suggest that *Ecklonia* should instead be placed in Arthrothamnaceae). In this thesis, I focus on South African *M. pyrifera* and *E. maxima*. *Macrocystis pyrifera* was selected as the focal point of this thesis as little research has been conducted on its South African populations. Furthermore, the use of *M. pyrifera* within the Chilean abalone industry and its unique properties for cultivation (Westermeier et al., 2013, 2016; Fleischman, 2016), make it an interesting candidate for aquaculture research. *Ecklonia maxima* was chosen as a suitable comparative species because it co-occurs with *M. pyrifera*, sharing similar environments, and is the most commonly used kelp feed in the South African abalone aquaculture industry. *Ecklonia radiata* and *L. pallida* were not used as comparative species in this thesis, due to a lack of overlap in the distributions of *E. radiata* and *M. pyrifera*, and while *L. pallida* does occur along the west and south-west coasts, its distribution in this area is complex, being dominant in the deeper water (>5 m depth) along the distribution of *M. pyrifera*.

*Ecklonia maxima* dominates inshore regions along the south-west coast of South Africa, making up approximately 50 % of the total seaweed biomass along the west coast (Velimirov et al., 1977; Field et al., 1980a; Rothman et al., 2006). This species is recorded as being distributed from just west of Cape Agulhas, South Africa (although, small patches of *E. maxima* exist 70 km east of Cape Agulhas, at De Hoop Nature Reserve), to Hottentotspunt, 60 km north of Lüderitz, Namibia (Bolton et al., 2012; Rothman et al., 2017). Although dominant inshore in the south, populations of this species become reduced in the northern distribution, gradually being replaced by *L. pallida* (Rothman et al. 2017). Along its distribution, *E. maxima* forms extensive kelp forests, at depths of up to 10 m. Blades of the sporophyte float at the surface at high tide forming dense canopies at some locations.

Sporophytes of *E. maxima* are structured with a distinct holdfast, stipe and blades (Fig. 3). Thalli of this species can reach 17 m in length, with an elongated stipe thinning towards the base (Anderson et al., 1997; Stegenga et al., 1997). The stipe is hollow just below the primary blade, forming a large gas bulb/float, which provides buoyancy to the kelp. Stipe morphology ranges from short in shallow water individuals, to long and more flexible with increasing depth. The lamina of *E. maxima* is made up of a primary blade from which many secondary blades are produced bilaterally. The primary blade can reach 1 m in length (particularly in young kelp and is often shorter in adults), is smooth and dentate along the lamina margin (Stegenga et al., 1997). The lamina is ribbon-shaped to rhomboid for juveniles and adults respectively. The holdfast of *E. maxima* is described as large (up to 0.4 m in diameter) and conical in shape, made up of intertwined branching haptera (Stegenga et al., 1997).

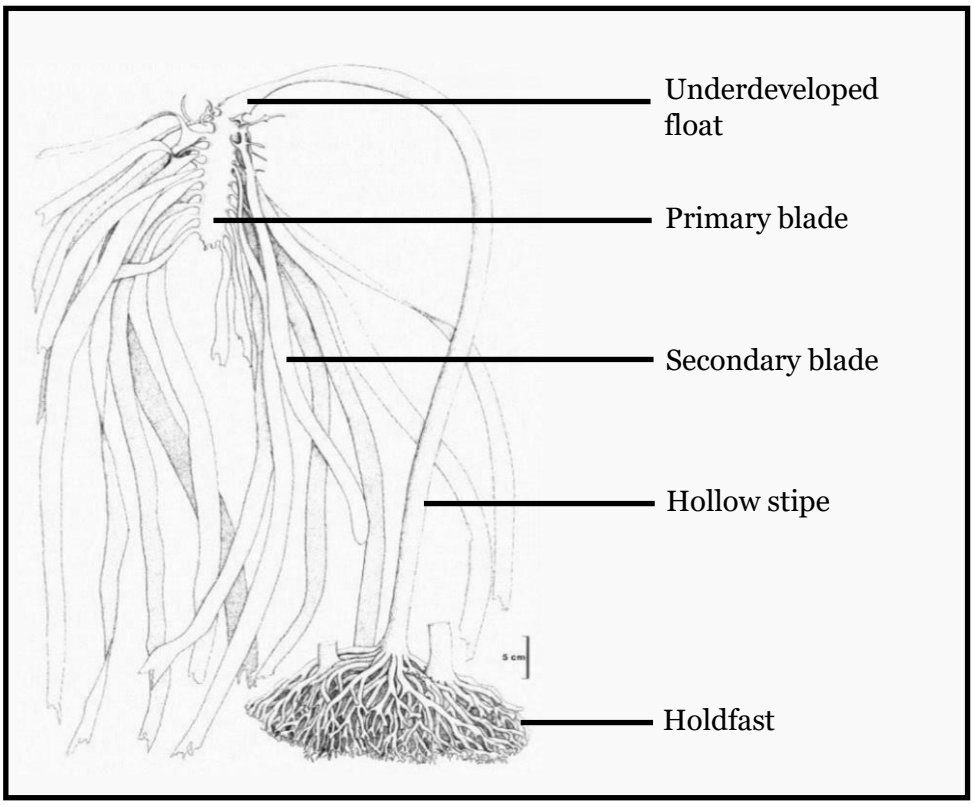


**Figure 3.** Diagram of a young *E. maxima* sporophyte (float not yet developed), showing the main structural components (Stegenga et al., 1997).

*Macrocystis* is a monospecific genus (Coyer et al., 2001; Demes et al., 2009; Macaya & Zuccarello, 2010) with a broad global distribution, occurring along the temperate west coasts of North and South America, New Zealand, southern Australia, Tanzania, South Africa and around many of the sub-Antarctic islands (Schiel & Foster, 2015) (Fig. 1). Within South Africa, *M. pyrifera* is distributed from Cape Point to Jacobsbaai, occurring in eight small known populations. It occurs in shallow, semi-enclosed bays, inshore of *E. maxima* forests (Papenfuss, 1942; Stegenga et al., 1997). The thalli of *M. pyrifera* in South Africa can reach up to 10 m in length (recorded at a population on the sheltered side of Robben Island, South Africa), but generally don't exceed 3 m on the mainland (Papenfuss, 1942; Stegenga et al., 1997; Fleischman, 2016). This makes

South African *M. pyrifera* shorter in comparison with other global *M. pyrifera* populations, which have been reported to reach up to 60 m in length (North, 1971).

The holdfasts of South African *M. pyrifera* can reach 0.5 m in diameter and are rhizomatous, made up of sub-dichotomously entangled terete branching haptera (Fig. 4). The holdfast is often fused from a number of individuals living close together. From the holdfast extends multiple terete stipes. An apical scimitar shaped blade (lamina) is present at the upper end of each stipe. This forms the point of blade separation in a secund series (Stegenga et al., 1997). The blades are positioned alternatively opposite along the length of the stipe and are ligulate in shape, tapering to a point and rugose, with regular marginal teeth (Coon, 1981; Stegenga et al., 1997; Schiel & Foster, 2015). A pneumatocyst (float) is found where the blade and stipe intersects and aids in keeping the stipe upright in the water column. Smooth sporophylls (reproductive blades) develop at the base, just above the holdfast (Stegenga et al., 1997). The stipe, blades and pneumatocysts collectively form an 'upright', which extends from the seafloor to the water surface, often forming dense canopies (Neushul & Haxo, 1963; Gerard, 1984). The little work that has been done on South African *M. pyrifera* was confined to physiological studies, which looked at nitrate assimilation and the polyphenolic distribution in *M. pyrifera* (Haxen & Lewis, 1981; Tugwell & Branch, 1989).

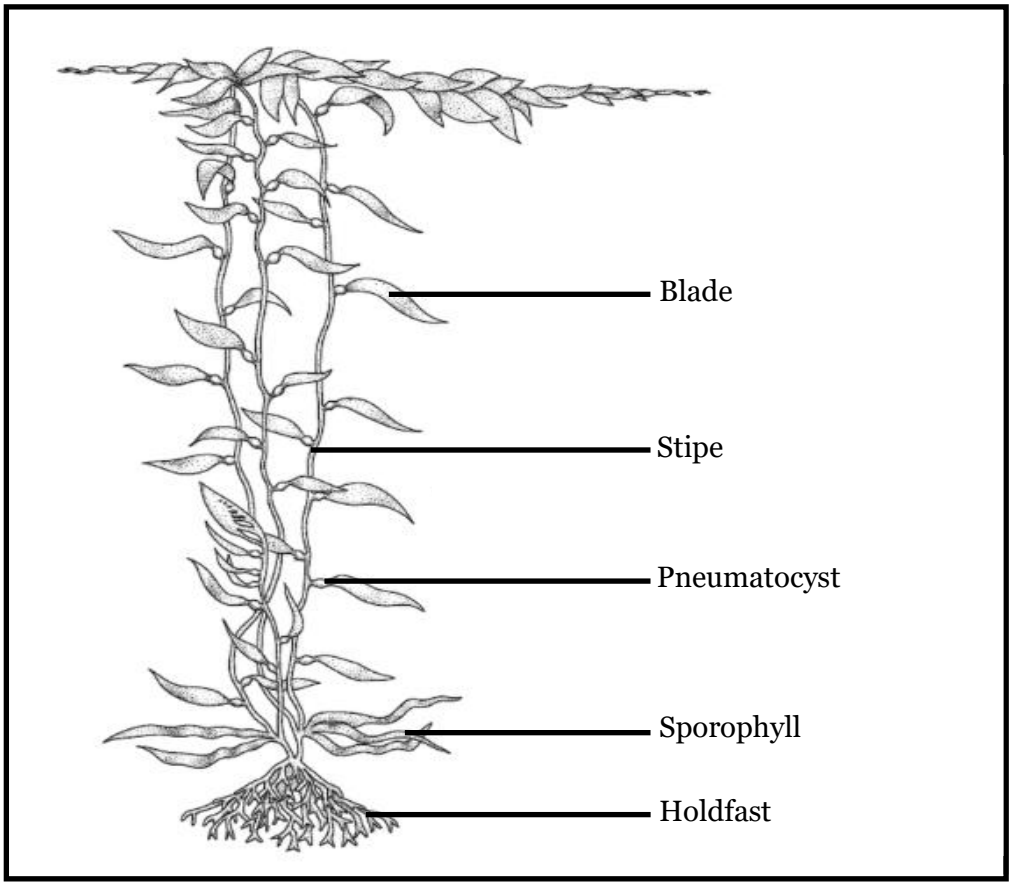


**Figure 4.** Diagram of an *M. pyrifera* sporophyte, showing the main structural components (Schiel & Foster, 2015).

The South African abalone aquaculture and kelp harvest industries

Initial attempts to cultivate abalone in South Africa began in the 1980's and developed throughout the 1990's, based on the aquaculture of the abalone, *Haliotis midae* (Sales & Britz, 2001; Naidoo et al., 2006; Bolton et al., 2013). The South African industry has since grown rapidly and is considered globally competitive, producing a high value abalone product, which is predominantly exported to Asian markets. Numerous abalone aquaculture farms are now established along the South African coastline, within which abalone are grown in highly specialised land-based grow-out operations (Troell et al., 2006; DAFF 2016a).

*Haliotis midae* have a flattened spiral shell, with a row of small holes running along the left side (Fig. 5). Under the front shell margin a gill cavity exists, into which water flows and exits via the holes. As abalone grow and the shell increases in size, new holes are formed and old ones close. Beneath the shell, a large muscular foot grips the substratum and allows for locomotion. Sensory tentacles and smaller projections extend from the foot beneath the shell and aid in detecting predators and food. Adult *Haliotis midae* in particular, can be identified by the large irregular corrugations, which run parallel to the growing edges (Muller, 1986; Branch et al., 2010) (Fig. 5). *Haliotis midae* is naturally distributed from Cape Columbine on the west coast, to north of Port St Johns on the south-east coast (Fig. 6), where adults occupy exposed positions or crevices on shallow reefs (juvenile abalone tend to hide under boulders or beneath sea urchins) (Muller, 1986; Day & Branch, 2000; Branch et al., 2010; DAFF, 2016b).

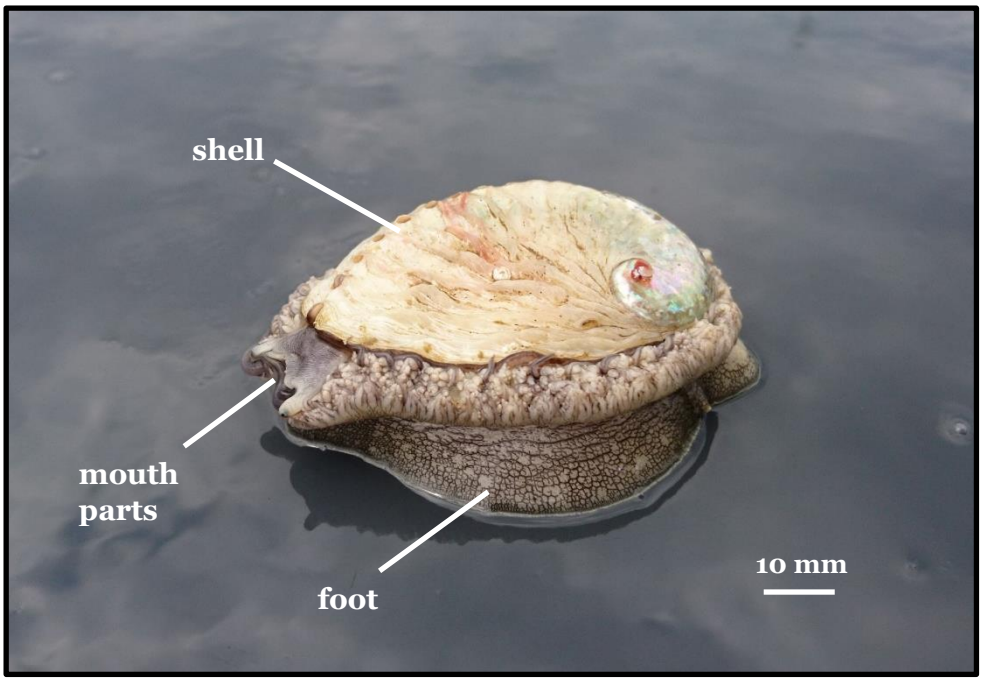


**Figure 5.** Young *Haliotis midae*, showing shell, mouth and foot parts. Picture taken during the feed inclusion trial.

Most South African abalone aquaculture farms are located along the south-west and western coastline (DAFF, 2016a). Many of these farms use freshly harvested *E. maxima* as a component of the abalone feed (Bolton et al., 2013). *Ecklonia maxima* is harvested by surface cutting and through beach-cast collection in commercial harvesting operations, from designated kelp harvest Concession Areas (Mark Rothman, Department of Environment, Forestry and Fisheries, pers. comm.). The South African coastline is divided into 23 Concession Areas (Fig. 6), where concessionaires can apply for the right to harvest a single seaweed functional group from within a specific Concession Area. Of the 23 Concession Areas, 14 contain kelp, with the dominant species being *E. maxima*. Within these Concession Areas levels of kelp harvesting are controlled through a Maximum Sustainable Yield (MSY) policy (Rothman et al., 2006; Troell et al., 2006; DAFF, 2016b).

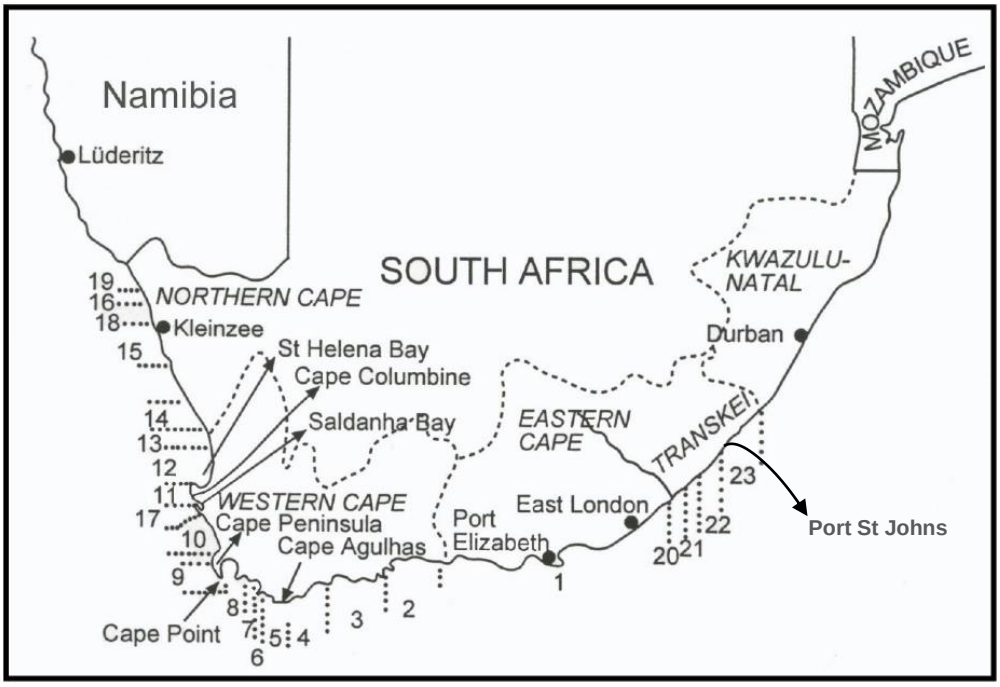


**Figure 6.** Map of South Africa, showing the distribution of *H. midae* from Cape Columbine in the west to Port St Johns in the east. The division of the coastline into the 23 seaweed harvest Concession Areas is also shown (Rothman et al., 2006).

The kelp harvest and abalone aquaculture industries are both viewed as economically important in South Africa, providing employment to coastal communities and generating economic gain through exports (Troell et al., 2006; DAFF, 2016a; Blamey & Bolton, 2018). For example, the fresh kelp harvested for the abalone feed sector has an estimated value of US$ 0.59 million.year$^{-1}$ (Blamey & Bolton, 2018). Moreover, the 2015 capital investment in the abalone aquaculture sub-sector was recorded at just above R 178 million (± US$ 13 million), making up approximately 67% of total investment inputs into South African aquaculture, and saw the highest increase in employment within the aquaculture sector (DAFF, 2016a).

Aims and objectives

At present the South African kelp harvest industry is well managed and even under-exploited in some areas (DAFF, 2016b). However, continual growth in the South African abalone industry and the farms that rely on this resource, will require increasing amounts of kelp (Anderson et al., 2006; Troell et al., 2006). Moreover, the ecosystem effects of large scale harvesting of South African kelp still requires further investigation (Anderson et al., 2006) and the location of available kelp may hinder the spread of the abalone industry along the South African coastline (Troell et al., 2006). These challenges provide motivation for research into alternative sources of abalone macroalgal feed for the South African abalone aquaculture industry. Here I investigated the use of *M. pyrifera* as an alternative source of supplementary abalone feed within the South African abalone industry. I hypothesised that *M. pyrifera* would provide similar growth outputs, when used as a feed for commercially grown *H. midae*, in comparison to fresh *E. maxima*. If *M. pyrifera* is found to be an effective supplementary abalone feed and sustainable sources of this resource are made available, it could serve as an alternative feed for the South African abalone industry.

Hobday & Pecl (2014) identified the waters off South Africa as a marine hotspot for climate change and has placed this region in the top 10 % of marine areas in the world that are experiencing the greatest rates of ocean warming. As has been seen in other global kelp forest ecosystems, climate change driven increases in ocean temperatures and MHW's will likely impact South African kelp forests in the future (Krumhansl et al., 2016). The loss of these kelp forests will impact the numerous industries dependent on this kelp resource, result in environmental degradation and see the loss of valuable ecosystem services (Blamey & Bolton, 2018). Here I investigated the impact of MHW's on two co-occurring South African kelp species, *E. maxima* and *M. pyrifera*. This was achieved by subjecting both species to an experimentally simulated MHW for a 72 hour period. I hypothesised that ocean temperatures exceeding 20 °C will severely impact the productivity of both species and result in kelp mortality. Understanding how these two species react to rising ocean temperatures and MHW's, will provide insight into how these two species will cope in a climate change future and whether we can expect changes to present South African *E. maxima* and *M. pyrifera* distributions.

Chapter 2-

*Macrocystis pyrifera* as an alternative feed in the South African abalone industry; effects on abalone growth and development, in comparison with *Ecklonia maxima*.

## Introduction

The first successful attempts at culturing abalone occurred in the 1960's in both the United States of America and Japan when abalone were successfully spawned and reared to adulthood. Since then, global abalone aquaculture has increased its market share, with subsequent declines in wild abalone fisheries. Technologies specialised for abalone aquaculture are now used in several countries, with the largest producers of abalone being China, Taiwan, Korea, South Africa, Australia, New Zealand, Chile, USA and Mexico (Flores-Aquilar et al., 2007; Hernández et al., 2009; Cook, 2014). Of the 130 Haliotidae species described, 14 are commercially exploited through aquaculture. Abalone have become a highly valued (US$ 35/kg) seafood product throughout the world. The greatest demand for abalone comes from within the Asian food market, where it is consumed as food (Sales & Britz, 2001; Cook, 2014; DAFF, 2016a).

Similar to the global abalone industry, the South African abalone industry is now dominated by land-based abalone aquaculture farms (DAFF, 2016a). South African abalone aquaculture is a fast-growing industry, whose success is largely attributed to cooperation between industry and research organisations within the country (Sales & Britz, 2001). Although six species in the genus *Haliotis* occur in South Africa, only *Haliotis midae* (Fig. 6) is farmed commercially (Sales & Britz, 2001). The first spat of juvenile *H. midae* was produced in 1981 and throughout the 1990's the large scale cultivation of *H. midae* began (Sales & Britz, 2001; Troell et al., 2006; Bolton et al., 2013). By 2001, 12 abalone farms were already operational, with an estimated value of

US$ 12 million (Naidoo et al., 2006). This increased to 18 farms by 2003, producing 462.02 t in that year (Naidoo et al., 2006; DAFF, 2016a). Growth within the industry continued, with a total of 1479 t abalone produced in 2015 (DAFF, 2016a). The South African abalone industry is now a strong competitor on global abalone markets and is economically important within South Africa, providing both employment and a high value export product (Troell et al., 2006; DAFF, 2016a).

*Haliotis midae* can reach a maximum size of approximately 200 mm over a 30 year period (Naidoo et al., 2006; DAFF, 2016b). However, South African abalone farms concentrate on growing abalone to a market-ready size of 100 mm (80 g) after 5 years (Sales & Britz, 2001; Troell et al., 2006). South African abalone farms pump seawater into land-based tank systems, which runs on an intensive flow through method. Some farms have both an abalone hatchery and facilities for growing abalone from seed to market size, while other farms purchase juvenile abalone from external hatchery facilities (Troell et al., 2006; DAFF, 2016a). Within hatcheries, young abalone are reared on diatom biofilms for several months and are then weened onto formulated feed and/or seaweeds at 4 - 6 mm shell length (5 - 6 months old) (Sales & Britz, 2001).

Optimising abalone aquaculture in South Africa is a key focus within the industry. A large aspect of this involves optimising the feeds given to abalone to ensure the best growth and end product (Naidoo et al., 2006; Troell et al., 2006; Bolton et al., 2013). Wild abalone are opportunistic herbivores, remaining inactive during the day and feeding at night. *Haliotis midae* have been noted to consume a wide range of macroalgal species in the wild, including *Ecklonia maxima*, *Ulva* and *Plocamium spp.*, and is believed to select algal species by their abundance in the local environment (Newman, 1968; Barkai & Griffiths, 1986; Stepto & Cook, 1993; Sales & Britz, 2001).

Abalone require a balanced diet of nutrients, with protein content considered to have a large influence on abalone growth (Britz & Hecht, 1997). For example, Britz (1996b), found that feeds with a higher protein content resulted in increased abalone growth. Soon after, Britz & Hecht (1997) determined that abalone of different sizes require protein in differing levels, with larger abalone having higher protein requirements. Apart from feed content, factors such as temperature and abalone density have also been found to influence abalone growth. For example, Britz et al. (1997) determined that 12 – 20 °C was the physiologically optimal range for growing *H. midae*, with growth rate and feed consumption increasing significantly with increasing temperature, peaking at 20 °C. However, temperatures above this range resulted in a negative growth performance and feed consumption (Britz et al., 1997). Moreover, abalone of different sizes and ages have been recorded to grow at differing rates (Sales & Britz, 2001).

Almost all South African abalone farms occur west of Cape Agulhas along the west and south-west coasts (DAFF, 2016a). Farms along this coast initially used kelp as the primary source of abalone feed, but the development of formulated feeds in the early 2000's saw reductions in the harvest and use of kelp for feed (harvesting later increased, peaking in 2011, but has since reduced). Formulated feeds (they have a high nutritional content (high protein) and low Feed Conversion Ratio (FCR)) are used largely because they decrease the amount of fresh kelp required to rear abalone to adulthood, thus reducing production costs (Britz, 1996a; Troell et al., 2006). The FCR is the efficiency with which a particular feed is converted by abalone into growth (g dry feed consumed/ g wet weight gain) (Sales & Britz, 2001). Formulated feeds have also been shown to improve abalone growth rates (weight gain) and aid in the weaning of juvenile abalone from diatoms to fresh kelp (Sales & Britz, 2001; Troell et al., 2006;

Bolton et al., 2013). Despite the apparent benefits of a formulated feed only diet, farmers still prefer to feed kelp or both kelp and formulated feed for two reasons: formulated feed only diets result in a higher incidence of sabellid infection and kelp is shown to provide better shell growth in abalone > 50 mm shell length (Troell et al., 2006). Therefore, many west and south-west coast abalone farms still use a combination of formulated feeds and freshly harvested kelp (fresh kelp has been shown to provide better abalone growth performance and is preferred by abalone, in comparison with dried kelp) (Naidoo et al., 2006; Troell et al., 2006; Anderson et al., 2007; Bolton et al., 2009).

West coast farms that use fresh kelp for abalone feed predominantly use *Ecklonia maxima*, a species found throughout the south-west and west coasts (Troell et al., 2006; Bolton et al., 2009, 2013). The commercial harvesting of fresh *E. maxima*, occurs in designated harvesting Concession Areas. The kelp species *Laminaria pallida* is also collected commercially, but it is not commonly used as abalone feed on the south-west coast. However, *L. pallida* is used in greater amounts north of Cape Columbine and in southern Namibia, due to it gradually replacing *E. maxima* in the inshore region. *Ecklonia maxima* thus provides the majority of the abalone kelp feed biomass along the west and south-west coast, making it an economically important resource for the growing South African abalone industry (Rothman et al., 2006, 2017; Troell et al., 2006; Anderson et al., 2007; DAFF, 2016b).

Both *E. maxima* and *L. pallida* have been used commercially since the 1950's, initially as beach cast, where the material was dried and exported overseas for alginate extraction (Rothman et al., 2006; DAFF, 2016b). Subsequently, South African kelp were harvested and processed into liquid growth stimulants for agricultural crops

(Anderson et al., 2003; Troell et al., 2006; DAFF, 2016b). The harvesting of kelp for abalone feed has grown immensely over the last 20 years due to increasing farm numbers and higher demands for kelp feed. Kelp harvested for abalone feed has increased from less than 1 t in 1992 to over 7000 t in 2011 (Rothman et al., 2006; Mark Rothman, Department of Environment, Forestry and Fisheries, pers. comm.). Harvesting has since reduced and it is estimated that just over 4000 t fresh kelp is currently harvested annually (Mark Rothman, Department of Environment, Forestry and Fisheries, pers. comm.).

Abalone require around 7 % of their body mass in kelp per day, due to the low protein content of kelp. For example, 5 t of fresh kelp daily is required to grow 100 t of abalone to 50 – 70 mm shell length. The high feed requirements of abalone, coupled with the growing demand for kelp (due to growth in the industry), will likely see increased levels of fresh kelp harvesting on South African kelp beds (Rothman et al., 2006; Troell et al., 2006). This may have negative consequences for South African kelp forest ecosystems, as the impacts of harvesting may persist for several years. This is because the commonly used harvesting technique involves the removal of the entire *E. maxima* kelp head (only the fronds are used for abalone feed), leaving the stipe and holdfast to die (Anderson et al., 2006; Rothman et al., 2006; Troell et al., 2006). As a result, the recovery of kelp within Concession Areas requires the recruitment of new sporophytes, which can take around 2.5 - 3 years (Anderson et al., 2006). Additionally, Anderson et al. (2006) found that while *E. maxima* recovered after 2.5 years, it took 4.5 years for its three obligate red algal epiphytes to recover post-harvest, meaning that ecosystem components can take several more years to recover. Therefore, determining alternative sources of abalone feed could prove beneficial to the future abalone

industry, allowing for continued growth in this sector, as well as reducing potential harvesting impacts on South African kelp forests.

Chile is one of the leading mariculture producers in the world, with the amount of fish, mussels, seaweed and other organisms produced increasing from 361,000 t in 1998 to 870,000 t of product produced in 2010 (Buschmann et al., 2008,2014). Chile began commercially farming the red abalone, *Haliotis rufescens* Swainson, in 1992. Most abalone farms in Chile are land-based grow-out operations, where abalone are fed on a mixture of macroalgae, predominantly the kelp species *Macrocystis pyrifera* (harvested from natural populations) and formulated feeds (Flores-Aquilar et al., 2007; Buschmann et al., 2008; Hernández et al., 2009; Camus et al., 2018). Abalone production in Chile has grown significantly over the last two decades, increasing from 73 t in 2001 to 840 t in 2011. This has been followed by an increase in the demand for fresh kelp, to supplement the needs of the growing abalone industry (Buschmann et al., 2014). Moreover, it has stimulated research into the potential cultivation of *M. pyrifera*, with many pilot scale trials taking place (most of which are based on growth from spores) (Macchiavello et al., 2010; Westermeier et al., 2013, 2016; Camus et al., 2018; Correa et al., 2016; Camus & Buschmann, 2017b). Despite the high research interest, macroalgal cultivation in Chile is still in its infancy, and so naturally harvested *M. pyrifera* continues to serve as an important source of feed biomass in the Chilean abalone aquaculture industry (Correa et al., 2016; Camus & Buschmann, 2017a).

Within South Africa, *M. pyrifera* is rare, existing as eight small populations, distributed from Cape Point to Jacobsbaai on the west coast (Gifkommetjie, Kommetjie, Oudekraal, Mouille Point, Robben Island, Melkbosstrand, Dassen Island and Jacobsbaai) (Stegenga et al., 1997; Anderson et al., 2007, pers. obs.) (Fig. 7). These

populations exist within *E. maxima* populations, commonly in shallow enclosed bays and typically grow to lengths of 2 - 3 m (Fleischman, 2016). Although not very abundant (± 200 km population range), research into the utilisation of *M. pyrifera* within the South African abalone industry may provide insight into its potential use as an alternative feed within the growing industry, as well as stimulate future research into *M. pyrifera* ecology and aquaculture in South Africa. This kelp species has already been shown, both locally (Fleischman, 2016) and abroad (Westermeier et al., 2013), to have the potential to be cultivated through vegetative propagation from holdfast material. No studies have yet to assess the effectiveness of *M. pyrifera* as a source of supplementary abalone kelp feed. If a sustainable source of *M. pyrifera* is made available, *M. pyrifera* could serve as a new alternative abalone feed and promote continued growth in the abalone industry.

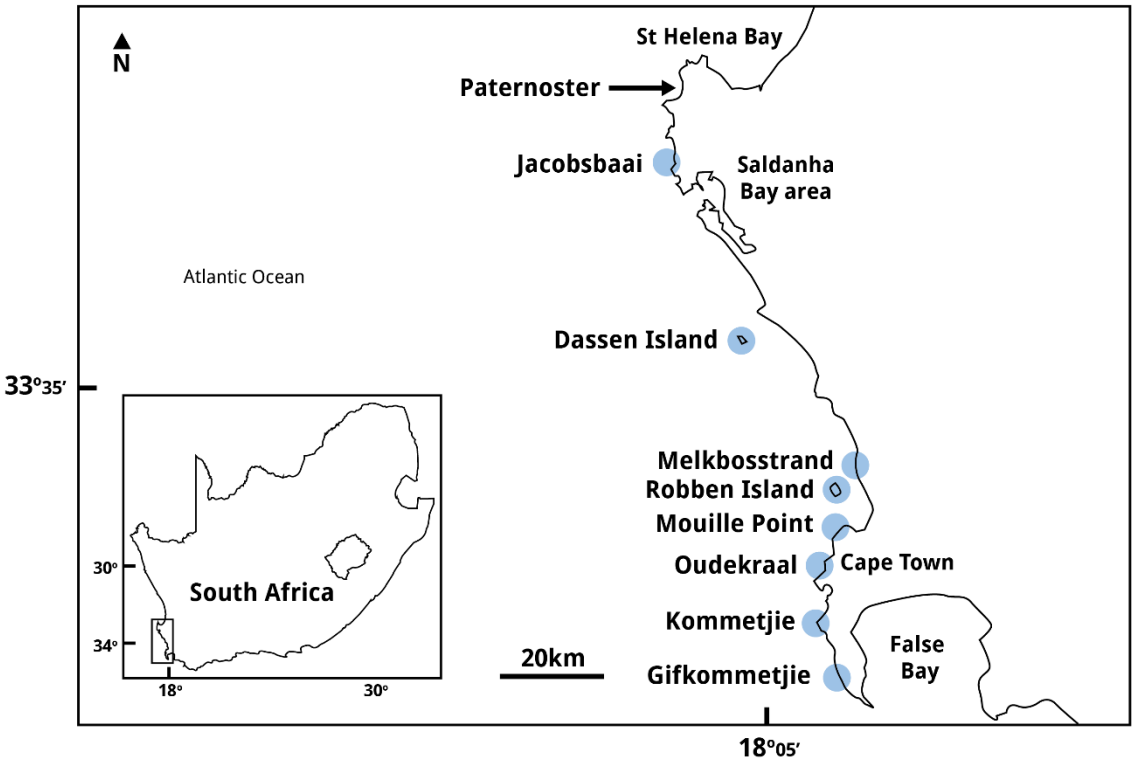


**Figure 7.** Map showing the locations of the eight *M. pyrifera* populations (blue circles) at the southern end of the South African west coast and the location of Paternoster, the site where the feed inclusion trial took place.

In this study, *H. midae* were grown at a commercial scale in a land-based grow-out operation at an abalone hatchery in Paternoster, South Africa. A feed inclusion trial was conducted to investigate two diets- (1) formulated feed supplemented with fresh *M. pyrifera*; (2) formulated feed supplemented with fresh *E. maxima*. This trial was conducted with the aim of determining, (1) if *H. midae* consumes *M. pyrifera* and (2) how the growth of abalone fed on a diet of formulated feed and *M. pyrifera* compares with those fed with formulated feed and *E. maxima*. I hypothesised that *H. midae* would feed on a supplemented *M. pyrifera* and formulated feed diet and would present similar growth rates, to those fed with formulated feed supplemented with *E. maxima*. This is so assumed due to the broad diet and the opportunistic feeding habits of *H. midae*, and the fact that *M. pyrifera* is broadly used as the primary source of feed in the Chilean *H. rufescens* aquaculture industry.

Materials & methods

To determine whether *Macrocystis pyrifera* could serve as an alternative feed for the abalone species, *Haliotis midae*, within the South African abalone aquaculture industry, a pilot scale feed inclusion trial was carried out. In this trial, the effectiveness of *M. pyrifera*, as an abalone feed, was compared with *Ecklonia maxima*- the fresh kelp feed predominantly used on abalone farms along the south-west and west coasts of South Africa. Both kelp feeds were fed in combination with a formulated feed.

Experimental setup

The abalone feed inclusion trial was carried out at a commercial abalone hatchery in Paternoster, Western Cape, South Africa (32° 48' 40" S, 17° 53' 40" E). The trial took place between June and December, 2017. Two commercial fiberglass abalone holding tanks (6 x 1.8 x 0.8 m; length, width, depth) were used for the trial setup (Fig. 8). The

holding tanks were positioned next to one another and were located in an open area. Seawater was provided at one end of each tank, with a mean water temperature of 11.95 ± 1.32 °C (range 10.3 – 15.7 °C) and at a flow rate of 3500 $L.h^{-1}$. The seawater was well aerated throughout the tank, via a basal aeration system, which bubbled air consistently throughout the tank.

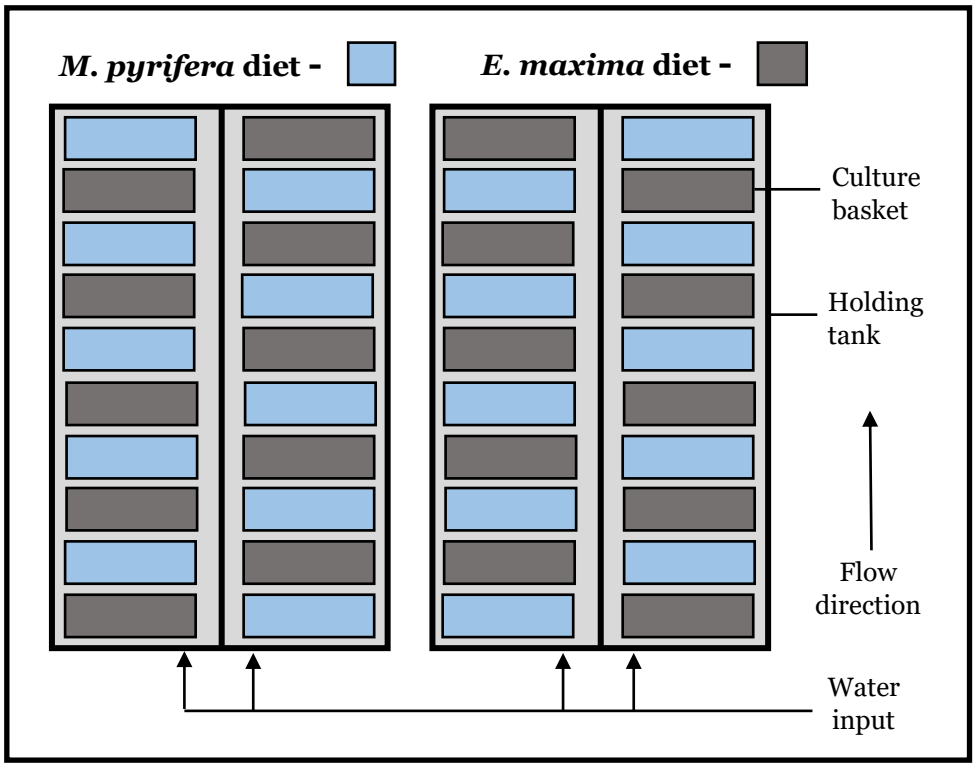


**Figure 8.** Abalone holding tanks, showing their positioning, placement of culture baskets, the separation of dietary treatments and direction of water flow.

The abalone were grown in plastic culture baskets (85 x 50 x 55 cm; length, width, depth), with 5 mm vertical slits (spaced roughly 10 mm apart) on all sides and holes on the bottom, to allow air and water to pass through (Fig. 9). Each culture basket contained vertically-orientated feeding plates, to increase available surface area. This is a commercially used setup, which allowed for a consistent flow of water through the baskets and created suitable area for the abalone to feed. A protective cover was placed over each culture basket, preventing predation by birds and shielding the abalone from

stressful environmental conditions. The protective cover also simulates low light conditions to promote feeding. Each holding tank contained two rows of ten culture baskets (20 culture baskets per tank) (Fig. 8) that were suspended above the bottom by ridges on each side of the holding tanks (Fig. 9). For identification, the culture baskets were labelled with a unique number for the duration of experiment.

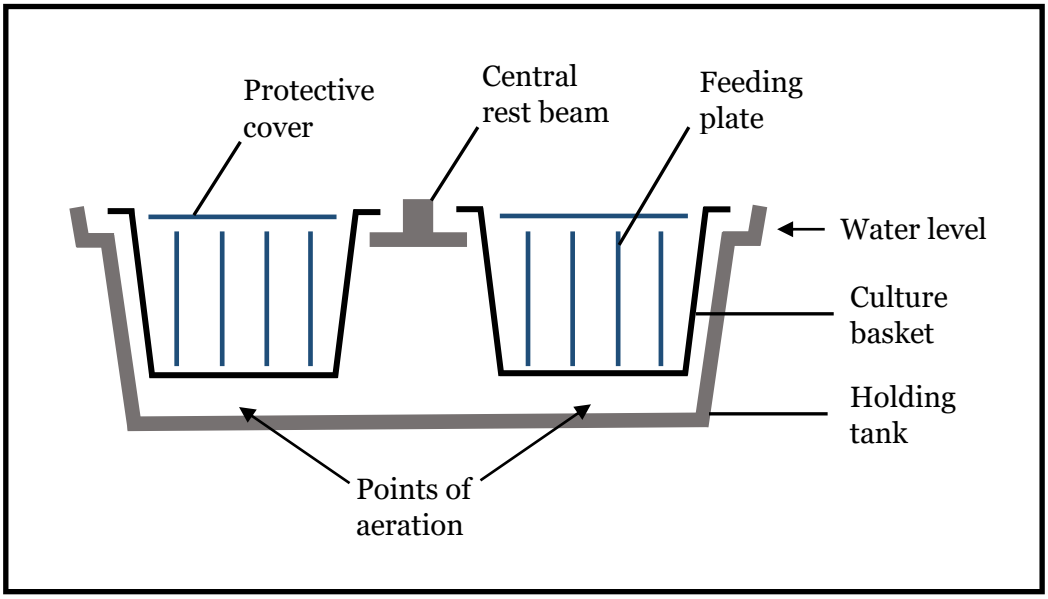


**Figure 9.** A cross section of an abalone holding tank, showing the placement of culture baskets, protective cover, feeding plates, water level and points of aeration.

Animals and diets

Abalone were provided by Buffeljags abalone farm (34° 44' 55" S, 19° 36' 33" E). All abalone provided were graded within the 45 - 60 mm shell length size class (Initial mean shell length and body weight for each treatment respectively: *M. pyrifera* + SAF 3000® = 47.01 ± 0.26 mm, 24.99 ± 0.44 g; *E. maxima* + SAF 3000® = 47.60 ± 0.22 mm, 24.98 ± 0.42 g); and were approximately 22 – 28 months old prior to being transported (approx. 300 km distance from Buffeljags to the Paternoster abalone hatchery). A total of 18,000 abalone were provided and evenly divided among the culture baskets, giving a stocking density of 450 animals per basket. Prior to the start

of the trial, the abalone were allowed to acclimatise in this environment for two weeks and were fed a diet of *E. maxima* and SAF 3000®. SAF 3000 is a formulated feed product produced by Specialised Aquatic Feeds ("SAF') (Pty) Ltd. SAF 3000 has an approximate protein content of 35 % (moisture- 12 %, crude fat- 2 %, crude fibre- 1.5 % and phosphorus- 0.08 %) and contains fishmeal, maize, starch, soya oilcake and various essential vitamins and minerals . Each holding tank served as a replicate for the experiment (two replicates), with 4,500 animals total per replicate, per dietary treatment (Fig. 8). Only two experimental replicates were used in this trial, due to financial and site constraints. The logistical limitations of having so few replicates are taken into consideration throughout this study and the sampling methods were designed to make up for the lack of replicates and provide reliable data.

At the start of the trial, 25 haphazardly chosen abalone from each basket were weighed (0.01 mg), using a portable electronic scale, and shell length (0.01 mm) measured along the longest axis, using electronic callipers. These data were used to calculate the mean abalone weight, which was then used to calculate the amount of fresh kelp (0.01 kg) and SAF 3000® (0.01 g) required per culture basket for the first month. Fresh kelp and SAF 3000® was fed at an abalone body weight ratio of 3.5 % and 0.6 %, respectively, according to standard farm practice at Buffeljags abalone farm.

Two separate diets were tested in this feed inclusion trial; (1) *M. pyrifera* + SAF 3000® and (2) *E. maxima* + SAF 3000®. Ten culture baskets from each holding tank were assigned to be given one of the two dietary treatments for the full duration of the feed inclusion trial (six months) (Fig. 8). The culture baskets of each holding tank were split into an alternating pattern, so that the culture baskets of each treatment would be spaced over the range of potential environmental conditions within the holding tanks

(Fig. 8). The *M. pyrifera* kelp feed was harvested from a nearby population in Jacobsbaai, South Africa and *E. maxima* was sourced locally and provided by the Paternoster abalone hatchery (Fig. 7).

Feeding and sampling procedures

Abalone were fed and baskets cleaned once a week. Prior to each feeding, the holding tanks were drained and all baskets were cleaned to remove waste and uneaten feed. During this time, the number of abalone that had died in each culture basket were noted and removed. The total remaining kelp in each dietary treatment was also weighed and removed. At the end of each month, 25 haphazardly chosen abalone from each culture basket, were weighed and shell length measured (with exception of the last month, where 35 individuals were selected from each basket, to broaden the data set). The subsequent monthly recorded weight values were then used to recalculate the amount of SAF 3000® and fresh kelp feed required by each culture basket for the following month. The amount of SAF 3000® and fresh kelp required by each basket was provided to the animals on a Monday and Thursday, respectively (formulated feed and kelp was provided separately to ensure continuous feed availability and eliminate the chance of preference for either feed type). This procedure was continued for a six month period, after which the abalone were returned to Buffeljags abalone farm.

Data processing

A Daily Increment in Shell Length (DISL in $\mu m.day^{-1}$) was calculated according to the following formula (Zhu et al., 2002):

$$DISL\ (\mu m/day) = [(SL_t - SL_i)/t] \times 1000$$

Where $SL_t$ is the final shell length, $SL_i$ is the initial shell length, and t is the number of days between the two measurements.

A Condition Factor value (CF in $g.mm^{-1}$) was determined using the following formula (Britz, 1996a):

$$CF = [BW\ (g) / SL\ (mm)^{2.99}] \times 5575$$

Where BW is the mean abalone body weight, SL is the mean abalone Shell Length and the values 2.99 and 5575 are constants (Britz, 1996a). The CF is a value that aims to explain the relationship between abalone weight and shell length. It gives an indication of whether the animal is gaining weight at the appropriate rate, in relation to increased shell length. A CF value close to or above 1 $g.mm^{-1}$ is desirable.

The abalone Specific Growth Rate (SGR in % body $weight.day^{-1}$) was calculated using the formula in Shpigel et al. (1996):

$$SGR = ((\ln(W_f) - \ln(W_i))/t) \times 100$$

Where $\ln(W_f)$ is the natural log of the final mean weight, $\ln(W_i)$ is the natural log of the initial mean weight and t is the number of days between the two measurements.

Statistical analysis

All data were expressed as means ± SE. Using a two-sample t-Test, no significant difference was detected among the two experimental replicates and thus the data were pooled. Using R (R Core Team, 2015), several two-way factorial analyses of variance (ANOVA) were carried out to determine whether the means for abalone body weight, shell length, DISL, CF and SGR, differed among the treatments and over the six month experimental period. Assumptions of homoscedasticity and normality were tested for in each case. Where significant, a Tukey post-hoc test was carried out to see where differences occurred. Lastly, two-sample t-Tests were performed to determine whether abalone $mortality.basket^{-1}.month^{-1}$ and the percentage of kelp feed consumed, differed between the two treatments. Results were considered statistically significant at the 95 % level ($p < 0.05$).

Results

Throughout the trial, abalone were observed feeding on the *Macrocystis pyrifera* feed provided (Fig. 10). The lamina of *M. pyrifera* was the fastest consumed component, with stipes commonly remaining in the basket at the time of cleaning. *Haliotis midae* fed with *M. pyrifera* supplemented feed increased in mean shell length from 47.01 ± 0.26 (M0) to 51.85 ± 0.31 mm (M6) over the six month experimental period. In comparison, the mean shell length of *H. midae* fed with *Ecklonia maxima* supplemented feed, increased from 47.60 ± 0.22 (M0) to 51.11 ± 0.35 mm (M6) (Tab. 1). Throughout the experimental period, no significant differences in mean abalone shell length, recorded at each month, were found between the two dietary treatments ($F= 7.230$, $df_1= 1$, $df_2= 224$, $p= 0.058$) (Tab. 1, Fig. 11). By month three, abalone in both dietary treatments displayed a significant increase in mean abalone shell length from the initial (M0) measurements ($F= 39.562$, $df_1= 6$, $df_2= 224$, $p< 0.05$) (Fig. 11). A significant increase in mean abalone shell length was again present in both dietary treatments, between the initial (M0) and final values (M6) ($F= 39.562$, $df_1= 6$, $df_2= 224$, $p< 0.05$) (Tab. 1, Fig. 11). Abalone fed with *M. pyrifera* and *E. maxima* supplemented feeds displayed a mean Daily Increment in Shell Length (DISL) of 31.19 ± 2.59 and 24.64 ± 2.31 $\mu m.day^{-1}$, respectively. No significant differences were found in mean abalone DISL between the two dietary treatments over the experimental period ($F= 0.853$, $df_1= 1$, $df_2= 192$, $p= 0.357$) (Tab. 1).

**Figure 10.** *Haliotis midae* attached to the underside of the plastic cover and feeding on the lamina of the *M. pyrifera* feed provided. Pictures were taken during the 6 month abalone feed inclusion trial.

Throughout the six month experimental period the mean abalone Condition Factor for both dietary treatments, remained above the value of 1 $g.mm^{-1}$ (Fig. 12). No significant increases or decreases in mean CF were observed between the initial (M0) and final (M6) values for both the *M. pyrifera* supplemented feed ($F= 4.014$, $df_1= 6$, $df_2= 224$, $p= 0.110$) and *E. maxima* supplemented feed ($F= 4.014$, $df_1= 6$, $df_2= 224$, $p= 0.221$). Furthermore, no significant differences in mean abalone CF were found between the two treatments at each month, with the exception of month two, where the mean abalone CF in the *E. maxima* supplemented feed treatment was found to be significantly higher than the *M. pyrifera* supplemented diet ($F= 4.014$, $df_1= 6$, $df_2= 224$, $p< 0.05$) (Fig. 12). The overall mean CF was recorded at $1.37 \pm 0.01$ $g.mm^{-1}$ for the *M. pyrifera* supplemented dietary treatment and $1.40 \pm 0.01$ $g.mm^{-1}$ for the *E. maxima* supplemented dietary treatment (Tab. 1).

**Table 1.** *Haliotis midae* abalone shell length growth parameters for each dietary treatment, measured over the six month experimental period. Different letters next to means represent statistically significant differences.

| Dietary Treatment | Mean shell length (mm ± SE) | | DISL (µm.day$^{-1}$) | | CF (g.mm$^{-1}$) |
|---|---|---|---|---|---|
| | Initial (M0) | Final (M6) | Range | Mean ± SE | Mean ± SE |
| *M. pyrifera* + SAF 3000® | 47.01 ± 0.26$^{a}$ | 51.85 ± 0.31$^{b}$ | 11.68 - 55.41 | 31.19 ± 2.59$^{a}$ | 1.37 ± 0.01$^{a}$ |
| *E. maxima* + SAF 3000® | 47.60 ± 0.22$^{a}$ | 51.11 ± 0.35$^{b}$ | 6.61 – 40.25 | 24.64 ± 2.31$^{a}$ | 1.40 ± 0.01$^{a}$ |

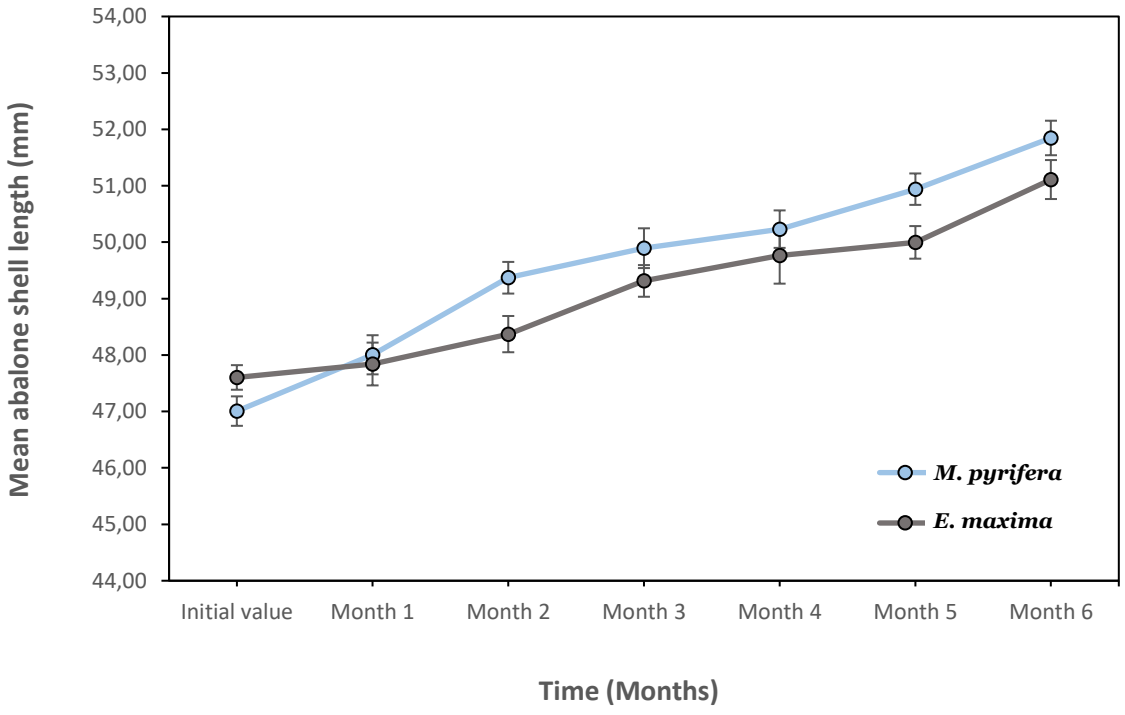


**Figure 11.** Mean abalone (*Haliotis midae*) shell length, measured over the six month experimental period. Bars represent standard error.

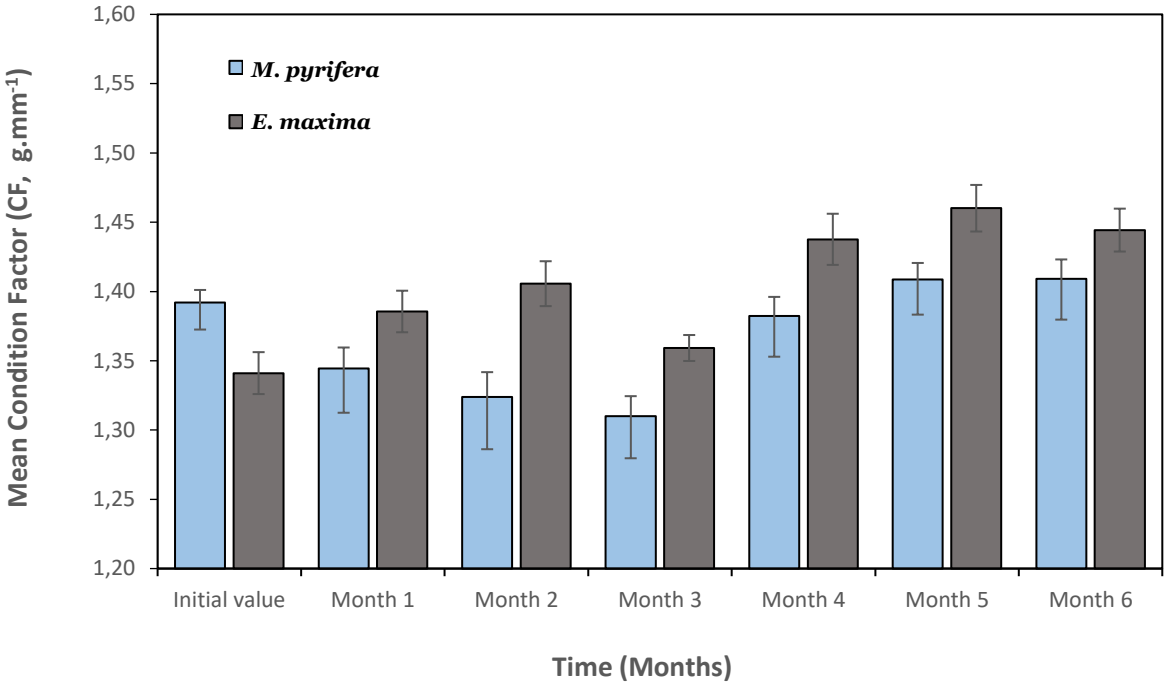


**Figure 12.** Mean Condition Factor (CF) of abalone (*Haliotis midae*), determined for each month of the six month experimental period. Bars represent standard error.

Over the course of the experimental period, abalone within the *M. pyrifera* supplemented dietary treatment increased from an initial (M0) mean body weight value of 24.99 ± 0.44 g to a final value (M6) of 33.89 ± 0.55 g, while mean abalone body weight increased from an initial value (M0) of 24.98 ± 0.42 to 33.26 ± 0.56 g (M6) within the *E. maxima* supplemented treatment (Tab. 2, Fig. 13). Similar to the mean abalone shell length results, no significant differences in mean abalone body weight at each month were found between the two dietary treatments over the experimental period ($F= 0.015$, $df_1= 1$, $df_2= 224$, $p= 0.902$) (Tab. 2, Fig. 13). At the end of month 3, both dietary treatments displayed significant increases in mean abalone body weight, when compared with initial (M0) mean abalone weight ($F= 63.993$, $df_1= 6$, $df_2= 224$, $p< 0.05$). Again, a significant increase was present between the initial

(M0) and final values (M6) for both dietary treatments ($F= 63.993$, $df_1= 6$, $df_2= 224$, $p< 0.05$) (Tab. 2, Fig. 13).

Throughout the experimental period, abalone fed with *M. pyrifera* and *E. maxima* supplemented feeds displayed mean Specific Growth Rates (SGR) of 0.19 ± 0.01 and 0.18 ± 0.01 % body weight.day$^{-1}$, respectively (Tab. 2). No significant differences were found in SGR, between the two dietary treatments, measured over the experimental period ($F= 0.180$, $df_1= 1$, $df_2= 192$, $p= 0.672$) (Tab. 2). No significant differences in abalone mortality rate were recorded between the two dietary treatments ($t(10)= 0.263$, $p= 0.798$). A mean mortality rate of 0.91 ± 0.26 mortalities.basket$^{-1}$.month$^{-1}$ was recorded in the *M. pyrifera* supplemented dietary treatment and 1.01 ± 0.27 mortalities.basket$^{-1}$.month$^{-1}$ recorded in the *E. maxima* supplemented dietary treatment (Tab. 3). These are very small mortality rates, with only 0.002 % of the 450 abalone per basket dying each month for both dietary treatments.

Additionally, it was found that the abalone samples consumed a significantly higher percentage of *E. maxima*, than they did *M. pyrifera* ($t(8)= 13.218$, $p< 0.05$). Abalone samples fed with *E. maxima*, consumed a mean value of 72.70 ± 1.26 % of the feed provided each month, while abalone samples given *M. pyrifera*, consumed a mean value of 31.76 ± 2.83 % of the feed provided each month (Tab. 3).

**Table 2.** *Haliotis midae* abalone body weight growth parameters of each dietary treatment, measured over the six month experimental period. Different letters next to means represent statistically significant differences.

| Dietary Treatment | Mean body weight (g ± SE) | | SGR (% body weight.day$^{-1}$) | |
|---|---|---|---|---|
| | Initial (M0) | Final (M6) | Range | Mean ± SE |
| *M. pyrifera* + SAF 3000® | 24.99 ± 0.44$^{a}$ | 33.89 ± 0.55$^{b}$ | 0.13 – 0.35 | 0.19 ± 0.01$^{a}$ |
| *E. maxima* + SAF 3000® | 24.98 ± 0.42$^{a}$ | 33.26 ± 0.56$^{b}$ | 0.10 – 0.26 | 0.18 ± 0.01$^{a}$ |

**Table 3.** Abalone mortality rate and feed consumption values for each dietary treatment, measured over the six month experimental period. Different letters next to means represent statistically significant differences.

| Dietary Treatment | Mortality rate (mortality.basket$^{-1}$.month$^{-1}$) Mean ± SE | Percentage kelp feed consumed (%.month$^{-1}$) Mean ± SE |
|---|---|---|
| *M. pyrifera* + SAF 3000® | 0.91 ± 0.26$^{a}$ | 31.76 ± 2.83$^{a}$ |
| *E. maxima* + SAF 3000® | 1.01 ± 0.27$^{a}$ | 72.70 ± 1.26$^{b}$ |

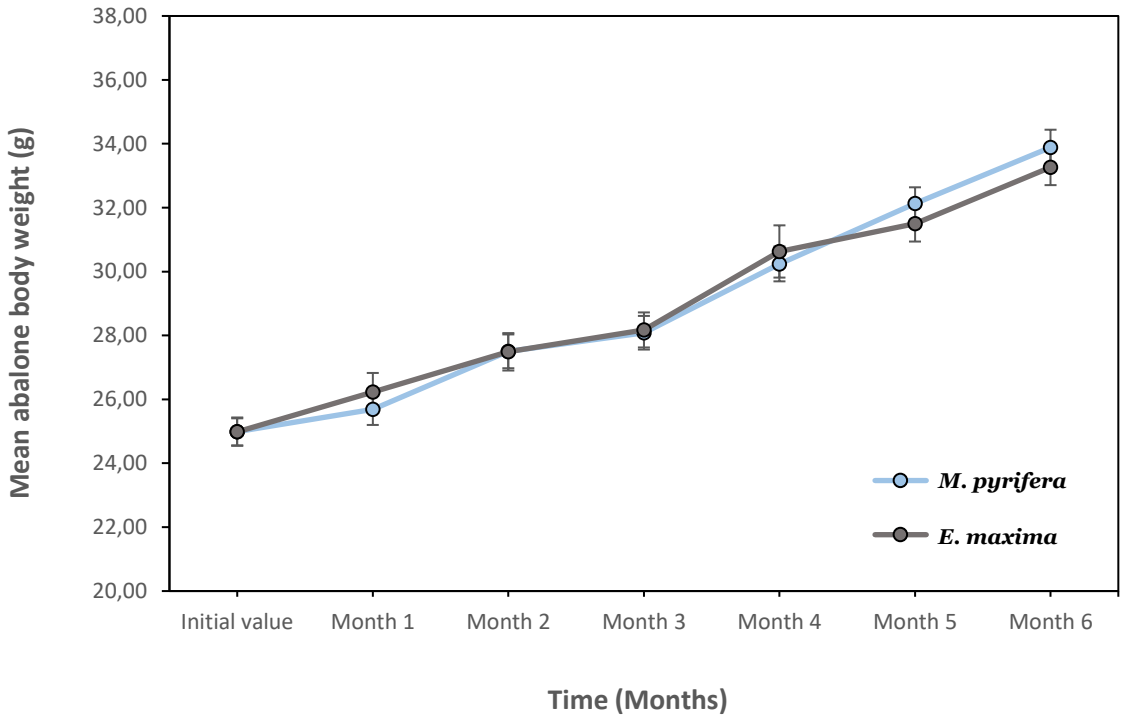


**Figure 13.** Mean abalone (*Haliotis midae*) body weight, measured over the six month experimental period. Bars represent standard error.

## Discussion

The type of feeds used to rear abalone from larva to marketable size contributes considerably to the effective aquaculture of this animal, and choice of feed is likely the most debated topic in abalone aquaculture. This study was carried out to establish whether South African *Macrocystis pyrifera* could serve as an alternative supplementary feed within the South African abalone aquaculture industry.

The findings of this study supported my hypothesis, as *Haliotis midae* was found to consume *M. pyrifera*. Additionally, the growth of *H. midae* fed a *M. pyrifera* supplemented diet, was found to be comparable to abalone fed on a diet of supplemented *Ecklonia maxima*.

The consumption of *M. pyrifera* by *H. midae* was expected. This species is known to be an opportunistic feeder and commonly feeds on drift kelp. Studies on *H. midae* have recorded this species grazing on several macroalgal species in the wild, often changing among algal species as they mature (Newman, 1968; Barkai & Griffiths, 1986; Stepto & Cook, 1993; Sales & Britz, 2001). Moreover, abalone have been found with at least two different species of algae in their gut at any one time (Barkai & Griffiths, 1986). South African *H. midae*, thus consumes a broad array of macroalgae and likely consumes many not yet tested algal species.

A large determinant of abalone growth and growth rates is the protein content of the feeds used (Britz & Hecht, 1997). Few studies have measured the protein content of *M. pyrifera* (and none have determined the protein content of South African *M. pyrifera*), however, a number of studies have measured *M. pyrifera* tissue nitrogen content. Using available tissue nitrogen data, it is possible to determine the approximate crude protein content by applying a Nitrogen-to-Protein Conversion (NPC) factor (Angell et al., 2016). Here I applied an NPC factor of 5, an updated universal NPC factor suggested by Angell et al. (2016), to studies that quantified *M. pyrifera* tissue nitrogen.

In northern Chile, Ortiz et al. (2009) determined local *M. pyrifera* to have an approximate crude protein content of 10.4 % dry weight (DW). In southern Chile, Buschmann et al. (2008) and Ravanal et al. (2017) measured *M. pyrifera* crude protein contents of 11.55 – 12.1 % DW and 11.5 % DW, respectively. In New Zealand, Fernández et al. (2017) measured tissue crude protein levels of approximately 10.35 % in local *M. pyrifera* populations. Along southern California, Zimmerman & Kremer (1986) found crude protein in *M. pyrifera* ranged 4.5 – 13.5 % DW, depending on the

time of year and whether an ENSO event was occurring. In comparison with these findings, unpublished work by Smith (2007), found the crude protein content of South African *E. maxima* to fluctuate between 10 – 12 % DW throughout the year. In addition to this, Dlaza et al. (2008) reported a protein range of 5 – 15 % and Francis et al. (2008b) a value of 9.05 % determined by proximate analysis on *E. maxima*. Looking at these values, one must acknowledge that nutrient content may vary along the length of a kelp and that ambient nutrient availability will largely impact tissue nitrogen concentrations, meaning that protein content is largely habitat specific. Therefore, the above values cannot be used to directly infer the protein content of the kelp species used in this study. Nonetheless, these values can suggest a range of protein levels for each species. Therefore, based on the information available, no large differences appear to exist in the maximum protein levels of *E. maxima* and *M. pyrifera*, in comparison with the high protein content provided by the SAF 3000® formulated feed (± 35 % protein). As a result, the similarity in protein content between the two kelp species might partially explain the lack of significant differences in the abalone growth rates recorded.

Despite similar growth rates, large differences in the consumption of the two kelp species were present. The percentage of *M. pyrifera* biomass consumed by abalone during the experimental trial was far less in comparison with the *E. maxima* consumed. A possible explanation could be differences in the concentration and distribution of polyphenols (chemical grazing deterrents) among the two species. However, analysis of polyphenols within South African populations of *M. pyrifera* and *E. maxima* by Tugwell & Branch (1989), found polyphenols to be present in greatest amounts in the meristematic and sporogenous tissues of both species, with relatively low levels in vegetative tissue. Additionally, the concentration of polyphenols in *E.*

*maxima* was found to be much greater in comparison with *M. pyrifera* (Tugwell & Btanch, 1989). During the feed inclusion trial no sporogenous *M. pyrifera* tissue was provided as supplementary feed, while abalone likely received *E. maxima* sporogenous tissue, due to sporogenous tissue being located on fronds. Therefore, due to a greater percentage of *E. maxima* being consumed during the trial period in comparison with *M. pyrifera*, the presence of polyphenols alone cannot serve as an explanation for differences in consumption between recorded between *M. pyrifera* and *E. maxima*.

Alternatively, differences in feed consumption may be explained by differences in either the Feed Conversion Ratio or other nutritional components between the two kelp species. This finding may have important implications for the abalone production cycle. For example, if *H. midae* fed with *M. pyrifera* requires less kelp biomass than if *E. maxima* was used, less energy would be expended to eat, leaving more energy available for abalone development. Moreover, far less kelp feed would be required to rear abalone to market size, providing economic benefits to the abalone farm. However, studies that measure the FCR and nutritional make-up of South African *M. pyrifera* are necessary to confirm these differences.

Nonetheless, the abalone performed well throughout the experimental period. Abalone fed with supplemented *E. maxima* increased in shell length by 6.87 % and body weight by 24.89 %. Abalone fed with supplemented *M. pyrifera* increased in shell length by 9.33 % and body weight by 26.26 %. It is difficult to compare the findings with those of other South African studies on *H. midae*, as other studies use abalone of varying initial sizes, differing stocking densities, different water temperatures and abalone feeds, all of which can impact the growth rates of abalone (Sales and Britz, 2001). For example, Naidoo et al. (2006) reported a mean DISL of 53 $\mu m.day^{-1}$ and an

approximate SGR of 0.39 % body weight.day$^{-1}$ for abalone fed with fresh *E. maxima* and Abfeed®, although water temperature was higher (15.5 ± 2.5 °C) and the mean initial abalone size was smaller (7.92 g, 34.75 mm). The results achieved in Naidoo et al. (2006) were similar to the growth rates recorded in this study. Differences in growth rates were likely the result of differences in experimental design and the lower temperatures recorded in this study (11.95 ± 1.32 °C).

Condition Factor values for both diets suggest that the abalone samples maintained a desirable body weight/ shell length ratio throughout the feed inclusion trial. Maintaining a suitable abalone CF is an important aspect of feed choice within the commercial industry, as this is an indicator of animal health and may impact the overall quality and value of the abalone product produced. Fluctuations in the CF values, between the months, can be attributed to changes in, among other factors, the physical conditions experienced during the trial and the structure of the feed inclusion trial (initial abalone size, stocking density and trial duration) (Sales and Britz, 2001).

The abalone samples in each dietary treatment were subjected to equal stocking densities, similar environmental conditions, were of a similar initial size and grew at roughly the same rate. Therefore, the levels of competition among samples and the availability of food, were more or less the same, accounting for the similarity in the mortality rates of both dietary treatments. The relatively low mortality rates experienced (0.002 %.basket$^{-1}$.month$^{-1}$), were likely due to ample feed being provided ('feeding to excess'), reducing the possibility of starvation, and the use of a protective cover, decreasing exposure to unsuitable conditions and predation by birds. Furthermore, as shown in Britz et al. (1997), the low water temperatures recorded throughout the trial may have also contributed to the low abalone mortality rates.

The methodology of this study could be improved by increasing the number of experimental replicates and increasing the length of the experimental feed inclusion trial. Increasing the number of treatments to include other algal species (particularly *Ulva spp.*, which is now cultivated and used as supplementary feed on some South African abalone farms (see Dlaza et al., 2008; Bolton et al., 2013, 2016)), or possibly a mixed diet treatment (see Naidoo et al., 2006) may also provide beneficial information. Lastly, assessing growth rates under differing amounts of kelp feed provided (as very little *M. pyrifera* feed was consumed) might also be useful. For example, Francis et al. (2008a) reported improved growth rates in *Haliotis midae* under a reduced *E. maxima* provision feeding regime. These improvements, would provide more reliable data, increased data for comparative purposes and show any potential data trends, which may only occur through seasonality or when long term growth rates are tested. These options were not possible in the present study due to time, site and financial constraints.

Nonetheless, this study explored a previously untested abalone feed in South Africa and provided baseline data for future comparison. These findings will hopefully stimulate further research into the use of *M. pyrifera* as an abalone feed in South Africa. Future use of this species should optimally be done in conjunction with other algal species, as mixed species diets have been shown to provide better abalone growth rates and ensure higher product quality (Day & Fleming, 1992; Simpson & Cook, 1998; Naidoo et al., 2006; Troell et al,. 2006).

Future research should determine other abalone feed parameters useful to industry such as the nutritional content and the FCR of South African *M. pyrifera*. This will assist in comparing *M. pyrifera* with other feeds and may provide insight into why this

feed was consumed in significantly smaller amounts, in comparison with *E. maxima*. Finally, due to the limited natural availability of *M. pyrifera*, investigations into the sustainable cultivation of *M. pyrifera* are essential to the future use of this resource.

This study showed that *H. midae* does consume South African *M. pyrifera*. Moreover, *H. midae* fed on a diet of SAF 3000® and fresh *M. pyrifera* presented growth rates no different from those fed with a combination of fresh *E. maxima* and SAF 3000®. If a sustainable source of *M. pyrifera* is obtained, this kelp species could serve as an alternative kelp feed in the South African abalone aquaculture industry.

Chapter 3-

Investigating the potential impact of ocean warming events on the productivity of two South African kelp species, the ecologically dominant *Ecklonia maxima* and the rare *Macrocystis pyrifera*

## Introduction

Anthropogenic climate change arguably poses the largest threat to the world's oceans. The increasing use of fossil fuels, human land-use and other anthropogenic activities have caused a rapid increase in atmospheric $CO_2$, from 280 part per million (ppm) in pre-industrial times, to the current value of 400 ppm (IPCC, 2007; Biskup et al., 2014; Shukla & Edwards, 2017). The effects of climate change are complex, but there is a general agreement that increasing atmospheric $CO_2$ has been accompanied by a rise in global air and ocean temperatures- the ocean has warmed by a global average of 0.11 °C per decade for the last 40 years (Biskup et al., 2014; Shukla & Edwards, 2017; King et al., 2018).

Rising ocean temperatures will increase climatic variability, leading to increases in the frequency, duration and intensity of extreme climatic events such as storms and Marine Heat Waves (MHW's) (Wernberg et al., 2013; Schlegel et al., 2017). The impacts of climate change and ocean warming are predicted to be most intense along the world's coastal and intertidal ecosystems, due to their high environmental variability, temperature extremes and other environmental stresses including wave action, excessive light, desiccation and osmotic stress (Pereira et al., 2015; Shukla & Edwards, 2017).

Understanding the implications of rising ocean temperatures is important as it is likely to affect the oceans ecological and physiological processes at all biological levels (Biskup et al., 2014). Temperature is known to be a major factor dictating global species distribution (Pereira et al., 2015). As a result, the most noticeable impact of a warming ocean will be changes in the distributions of marine organisms as they attempt to stay within their thermal tolerance ranges (O'Connor et al., 2012; Biskup et al., 2014; King et al., 2018). Changes in species distributions, due to ocean warming, commonly involves poleward shifts to cooler waters (Franco et al., 2018; King et al., 2018). Much evidence is already present showing the movement of coastal species, including species of kelp, to higher latitudes in many parts of the world including Europe, the east and west coasts of North America and many Southern Hemisphere locations, driven by rising ocean temperatures (Wernberg et al., 2010, 2011, 2016; Filbee-Dexter et al., 2016; Franco et al., 2018; King et al., 2018; Piñeiro-Corbeira et al., 2018).

Laminarian kelp are key ecosystem engineers, providing invaluable ecosystem services along 25 % of the world's temperate coastlines (Biskup et al., 2014; Blamey & Bolton, 2018; Filbee-Dexter & Wernberg, 2018). Global kelp populations are currently subject to a multitude of threats and have already been heavily altered by human activity (Filbee-Dexter et al., 2016; Franco et al., 2018). Findings suggests that 38 % of global kelp populations have declined over the last 50 years, with climate change and anthropogenic activities being the driving force behind reductions (Krumhansl et al., 2016; Filbee-Dexter & Wernberg, 2018).

Kelp (Laminariales) are temperate, sub-polar and arctic organisms, requiring cool, nutrient rich waters to thrive. Kelp are shown to display a hump-shaped thermal

performance curve, where the rate of biochemical reactions increase exponentially with increasing temperature and peaking at the optimal temperature of a species' thermal tolerance range. This is followed by a sharp decrease above the optimal temperature as biological components become damaged (Harley et al., 2012). For example, while respiration increases continuously with rising temperatures, photosynthesis has been shown to increase to a plateau (photosynthetic maximum) and then rapidly decline near the upper limit of the temperature tolerance range. Therefore, kelp are sensitive to increasing ocean temperatures, especially where temperatures exceed their thermal optima. High ocean temperatures can negatively impact kelp growth, survival, reproduction, recruitment and can result in damage to kelp tissue (cellular damage in kelp reduces tissue strength and extensibility, increasing vulnerability to wave forces (Simonson et al., 2015)) (Wernberg et al., 2010, 2013; Harley et al., 2012; Biskup et al., 2014; Filbee-Dexter et al., 2016; Muth et al., 2019). Rising ocean temperatures thus poses a threat to the persistence of kelp populations. Furthermore, rising temperatures can indirectly stress kelp populations through the alteration of epiphyte activity and herbivory (Krumhansl & Scheibling, 2011; Filbee-Dexter et al., 2016).

Warming has also been shown to have a varying impact on the different life stages of kelp, with strong influences on kelp early life stages (gametophytes and sporophyte recruits, including egg production) (Shukla & Edwards, 2017; Muth et al., 2019). For example, the effect of elevated temperatures on the early life stages of *Macrocystis pyrifera* was found to decrease germling production, male and female gametophyte growth and survival, and delay sporophyte recruitment and growth (Shukla & Edwards, 2017). In addition, Bolton & Anderson (1987) found that supra-optimal temperatures reduced growth and slowed egg release in female *Ecklonia maxima*

gametophytes, while sporophyte recruit relative growth rate decreased rapidly above 20 °C. The success of early life stages in kelp and the resilience of recruits is essential to the persistence of kelp populations further emphasising the threat of ocean warming and MHW's (Pereira et al., 2015). Loss of foundational kelp species will lead to changes in community makeup and disruptions to ecosystem functioning, with negative consequences for associated organisms and surrounding ecosystems (Johnson et al., 2011; Wernberg et al., 2013; King et al., 2018).

In response to rising ocean temperatures kelp are likely to display two survival strategies- a shift in distributional ranges or acclimatisation to new environmental conditions (Clarke, 1996; O'Connor et al., 2012; Shukla & Edwards, 2017; Franco et al., 2018). For example, recent studies carried out in Portugal (Biskup et al., 2014; Pereira et al., 2015) and the British south coast (King et al., 2018) already report on shifts in the distribution of local kelp species, showing a decrease in abundance of kelp at equatorial range edges and a poleward movement to cooler waters. However, a consequence of these movements are the vacant niches left behind, resulting in ecosystem degradation (King et al., 2018). To cope with changing environmental conditions and allow for continued functioning some species of kelp have been shown to acclimatise by changing their structure, altering physiological or metabolic processes (Davison, 1991). Nonetheless, there are limitations to kelp acclimatisation and changes to physiology and metabolism is a costly process, which can leave individuals susceptible to other stresses (Wernberg et al., 2010; Biskup et al., 2014).

The future of global kelp populations is thus dependant on their ability to change distributions or acclimatise. However, where range shifts are not possible, temperature increases are too rapid or temperatures exceed a kelp's physiological

threshold, direct mortality and local extinction is likely to occur (Clarke, 1996; Biskup et al., 2014; Pereira et al., 2015; Filbee-Dexter & Wernberg, 2018). Moreover, differing kelp species have been shown to react to rising temperatures and extreme warm events at differing levels, due to differences in thermal tolerance ranges (Biskup et al., 2014; Pereira et al., 2015, Muth et al, 2019; Wernberg et al., 2019). This results in some kelp species being more susceptible to local extinction than others. For example, kelp species from colder latitudes display ranges and temperature optima at lower temperatures than warmer latitude kelp species (Piñeiro-Corbeira et al., 2018).

There are already many reports of large scale loss of kelp populations, driven by ocean warming and MHW's (Johnson et al., 2011; Filbee-Dexter et al., 2016; Krumhansl et al., 2016; Filbee-Dexter & Wernberg, 2018). In many cases, the loss of a kelp population was followed by an influx of other warm water adapted algal species, including invasive and 'turf' forming macroalgae (Filbee-Dexter et al., 2016). For example, populations of *Ecklonia radiata* along Western Australia experienced a 100 km range contraction, followed by a rapid tropicalisation and regime shift to turf algae, due to decades of gradual warming along the coastline and an extreme MHW during the summer of 2010 – 2011. This event saw an increase in turfs from under 10 % to over 80 %, and a 43 % loss of local kelp forests over two years (Wernberg et al., 2013, 2016; Filbee-Dexter & Wernberg, 2018).

Turf dominated ecosystems are far less complex with minimal ecosystem benefits, when compared to kelp forests. These regime shifts also have consequences for humans, with resultant losses in tourism, recreational and commercial fisheries and other beneficial traits of kelp forests (Filbee-Dexter & Wernberg, 2018). Furthermore, turf regimes do not show recovery as they are reinforced by strong feedback

mechanisms preventing kelp recruitment and increasing turf resilience (Wernberg et al., 2016; Filbee-Dexter & Wernberg, 2018).

Along the west and south-west coast of South Africa, the Benguela current creates a temperate environment characterised by cool, nutrient rich waters and highly productive ecosystems (Stegenga et al. 1997; Blamey et al., 2015; Schlegel et al., 2017) (Fig. 14). Nutrients are brought to surface waters via a seasonal upwelling system, driven by a prevailing south-easterly wind regime (Stegenga et al. 1997; Schlegel et al., 2017; García-Reyes et al., 2018; Veitch et al., 2018). Upwelling impacts multiple environmental factors, including light and nutrient availability, temperature and turbidity; all of which influence the functioning of coastal ecosystems. The highly variable physical environment and presence of seasonal upwelling, drives a highly productive ecosystem unique to this coastline (Bolton & Levitt, 1987; Blamey at al., 2015).

In South Africa, overexploitation and spatio-temporal changes in key species, due to changes in the physical environment, are shown to have already impacted some of South Africa's marine ecosystems over the last three decades (Blamey et al., 2012, 2015, Blamey & Bolton, 2018). Many fragile ecosystems are under stress and shifts in important species have already been recorded. One of the most notable of these shifts is the eastward movement in the commercially important kelp species, *E. maxima* (Bolton et al., 2012; Reimers et al., 2014). In 2006, the distributional eastern boundary limit of *E. maxima* was found to have shifted more than 70 km eastward, around Cape Agulhas. This movement is believed to have been caused by upwelling intensification and coastal cooling, allowing this temperate species to extend its range (Rouault et al.,

2010; Blamey et al., 2012, 2015; Bolton et al., 2012; Reimers et al., 2014; Lamont et al., 2018).

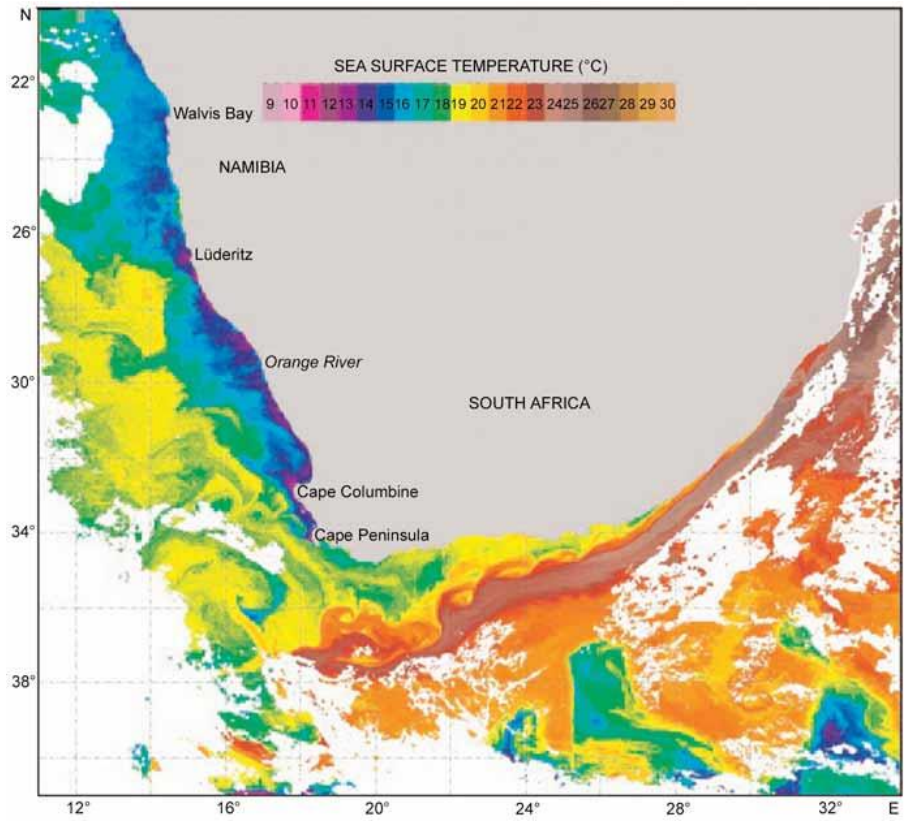


**Figure 14.** NOAA AVHRR SST image, taken in May 2003, showing the warm Agulhas current on the east coast and upwelling in the Benguela region, along the south-west and west coast of South Africa (Weeks et al., 2006).

Additionally, the incidence of MHW's are not uncommon off the coast of South Africa. Recent work by Schlegel et al. (2017), who analysed long-term nearshore *in situ* data at various South African sites and NOAA daily SST data, found near-shore MHW's to have occurred on average at least once per year along the east, south and west coasts of South Africa and that the frequency of these events were on the rise. West and south coast MHW events were found to be more frequent, intense and have a longer duration than east coast events (Schlegel et al., 2017). The recorded events lasted on average

between 7.7 - 10.9 days and saw anomalous increases in sea temperatures of between 1.72 – 2.65 °C, depending on the data set used (Schlegel et al., 2017).

*Macrocystis pyrifera* and *E. maxima* are two species that co-occur along the South African west coast, both with fronds at or near the surface. While *E. maxima* has a near continuous distribution from Lüderitz in southern Namibia to Cape Agulhas on the south coast of South Africa, *M. pyrifera* is currently known to exist only in eight small populations, between Cape Point and Jacobsbaai on the west coast (*ca.* 200km of coastline) (Fig. 7). Within its west coast distribution, *E. maxima* dominates the inshore region to a depth of up to 10 m, forming dense kelp forests. *Macrocystis pyrifera* exists as patches of individuals on rocky outcrops and large boulders within enclosed bays, often at a depth of 1 - 2 m; with the exception of the Robben Island population, where it lies in the shallows (1-2 m) but extends into deeper waters up to a depth of approx. 6 m (Derek Kemp, Department of Environment, Forestry and Fisheries, pers. comm.). These *M. pyrifera* populations are frequently bordered by and found in amongst *E. maxima* forests.

No studies have investigated the upper limit of thermal tolerance of South African *M. pyrifera* and although Bolton & Anderson (1987) examined the thermal tolerance range of extremely small *E. maxima* sporophytes (*ca.* 1 mm), none have investigated larger sporophytes of this species. Understanding how these two species will react to these anomalous warming events and in particular, MHW's, is critical to predicting the future functioning and resilience of these ecosystems. The limited distribution displayed by South African *M. pyrifera* makes this species particularly vulnerable to changing oceanographic conditions. Additionally, understanding the temperature limitations of *M. pyrifera* may also explain its current limited distribution. In contrast,

*E. maxima* has a widespread distribution along the entire South African west and south-west coasts, and also occurs in southern Namibia. *Ecklonia maxima* is a key resource in the South African abalone industry as fresh abalone feed. Changes in the present abundance of this species may have negative consequences for both the local industry and the ecosystems it supports.

In this study, *E. maxima* and *M. pyrifera* sporophyte recruits were subjected to a simulated MHW event. This was done with the aim of determining the impact of rapidly elevated ocean temperatures on the productivity of these two species. Although *Laminaria pallida* occurs along the South African west and south-west coasts, it was not used in this study, as it commonly dominates below 5 m depth, with corresponding different environmental conditions to the other two species (including temperature). Kelp survival and productive capacity was assessed by measuring kelp oxygen production post thermal stress. Although these species co-occur, *M. pyrifera* exists only in the extreme south of the west coast, while *E. maxima* is distributed more broadly and over a larger temperature range, with an eastern boundary near Cape Agulhas and sporadic patches occurring at De Hoop Nature Reserve. Therefore, I predicted that *E. maxima* and *M. pyrifera* would display differing levels of thermal tolerance, due to adaptation to differing past and present distributions, with *E. maxima* displaying a broader thermal tolerance range. However, I expected both species to show reduced oxygen production and survival at temperatures above 20 °C, as this temperature is considered the temperature threshold for many kelp species and neither *E. maxima* nor *M. pyrifera* presently exist in South African locations where monthly mean temperatures exceed this threshold. If this is the case, one can predict range contractions of both species into cooler waters, as a result of increasing frequency and intensity of MHW's.

Materials & methods

To determine the response of South African *Macrocystis pyrifera* and *Ecklonia maxima* to Marine Heat Waves, controlled thermal stress experiments were carried out. These experiments assessed the impact of a thermal gradient on the productivity of the target species, measured via kelp oxygen production.

Species distribution and collection site

For the purpose of this study all kelp sporophyte recruits were collected from Kommetjie (34° 08' 25" S, 18° 19' 14" E) (Fig. 7), South Africa, where *E. maxima* and *M. pyrifera* are found in close proximity to each other. By collecting recruits from the same site, individuals would have been exposed to similar photoperiods, water temperatures and other environmental conditions. In Kommetjie, *M. pyrifera* is found on rocky substratum in the immediate subtidal range. This population is usually covered by 1 – 2 m of water and is semi-exposed to wave action. Individual *E. maxima* and *L. pallida* sporophytes are occasionally present in openings in the *M. pyrifera* canopy. The *Macrocystis* population covers an area of approximately 250 $m^2$, with an *E. maxima* forest bordering the *M. pyrifera* population on the seaward side. The inshore limit of the *E. maxima* forest sits at a depth of approximately 2 m at high tide, increasing to 6 m at the outer limit of the population. Similarly, this population also exists on rocky substrate and is semi-exposed to wave action, making easy access to both these populations only possible during spring low tides.

Experimental design

The experiment was run separately for each species (*M. pyrifera* and *E. maxima)* over July/August 2018, due to logistical constraints. Twenty kelp recruits of each species, 20 - 40 cm in length, were collected from the Kommetjie site and placed immediately

in two 25 L containers, containing seawater. The recruits were transported to the University of Cape Town in under 2 hours and placed in the experimental laboratory, maintained at 11 °C. The recruits were cleaned, removing both sediment and epiphytes from the holdfast. The recruits were then placed into a single, well-aerated 100 L holding tank, filled with 0.2 μm filtered seawater and maintained at a control temperature of 15 °C - the intermediate value for the annual mean range for the west and south-west coast (Stegenga et al., 1997). The kelp recruits were then allowed to acclimatise for a period of five days to recover from collection and transportation stress. During this period the recruits were shifted around the tank twice daily to avoid edge effects. Throughout acclimatisation and the experimental period all seawater used was provided with Provasoli Enriched Seawater (PES) medium (Provasoli, 1968). PES was provided to create conditions without nutrient-limited stress. Kelp recruits were also exposed to a 12 h day photoperiod, with a photon flux density range of 100 – 120 $\mu mol.m^{-2}.s^{-1}$, using LIGHT WORX cool white LED, 7 WATT bulbs (AC 220- 240 V, 665 LUMENS, 6500 KELVIN), fitted into desk lamps. The light provided simulated the approximate light levels available to juvenile kelp in the understory (Gerard, 1984; Anderson & Bolton, 1985; Clark et al., 2004).

After acclimatisation, individual recruits were placed into clear one litre plastic sample jars, each filled with 0.2 μm filtered seawater and aerated using aeration stones (Fig. 15). The 20 sample jars were then split and labelled according to four temperature treatments- 15 (control), 17.5, 20 and 22.5 °C, with five replicates per treatment. Each of the five replicate jars were then placed into one of four clear plastic water baths (Fig. 15). Each water bath was filled with fresh water and maintained at one of the four corresponding treatment temperatures using a LABCON™, CPM50 water heating unit (Fig. 15). The water in each bath was circulated to ensure even temperatures

throughout. The recruits remained in these conditions for 72 hours, simulating a MHW.

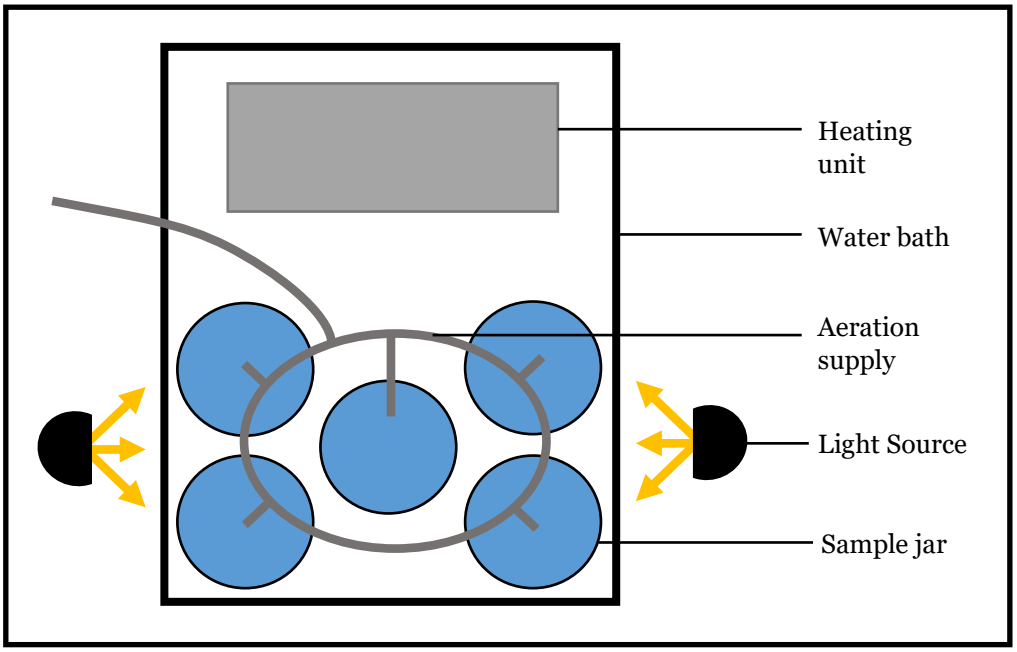


**Figure 15.** Top view of the experimental setup, showing the water bath, position of sample jars in the water bath, location of light sources and aeration supply.

Oxygen yield determination

After three days all water baths were cooled down to the control temperature (15 °C), to create a non-stressful environment. The water in each sample jar was then replaced with low oxygen 0.2 µm filtered seawater. The low oxygen seawater was created by bubbling nitrogen gas, derived from liquid nitrogen, through seawater. An initial dissolved oxygen reading in $mg.L^{-1}$ was taken using a CyberScan DO 300 Series, waterproof hand-held Dissolved Oxygen/Temperature meter (EUTECH instruments). Each of the sample jars was then sealed and left for one hour. After this time, the dissolved oxygen in each sample jar was measured to 0.01 $mg.L^{-1}$.

The kelp recruits were then removed from their sample jars and photographs of each recruit were taken. Additionally, thallus (lamina, stipe and holdfast) deterioration (loss of tissue and discolouration) in the recruits was also noted (this was expressed at the percentage of recruits showing thallus deterioration for each temperature treatment). The recruits from each treatment were then dried in an oven, set at 45 °C, over 48 hours (Fig. 16). After this period, the dry weight (DW) of each recruit was measured using a portable scale (0.01 mg). The dry weight was then used to calculate the rate of oxygen production for each recruit in mg $O_2$.g $DW^{-1}$.$hour^{-1}$.

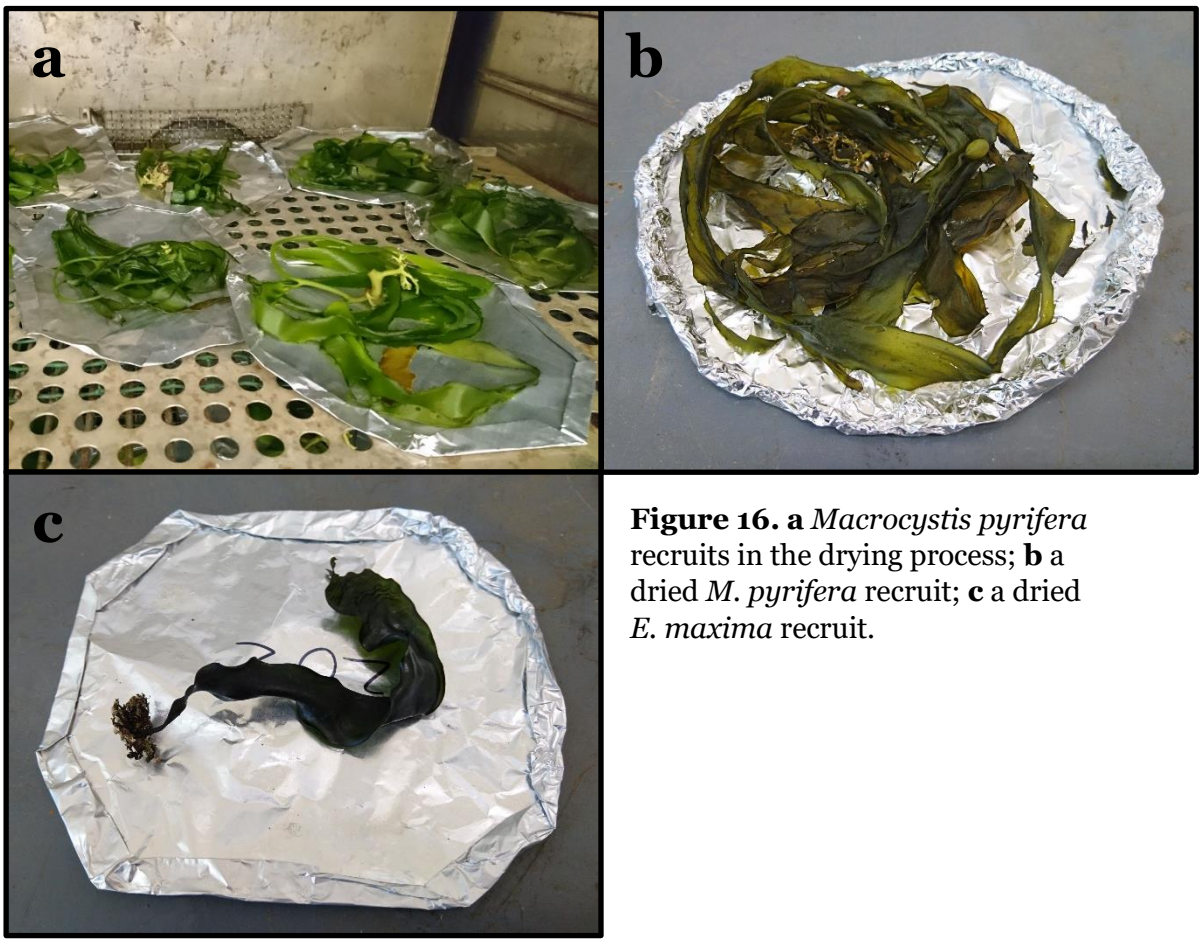


**Figure 16. a** *Macrocystis pyrifera* recruits in the drying process; **b** a dried *M. pyrifera* recruit; **c** a dried *E. maxima* recruit.

Statistical analysis

All data were expressed as means ± SE. Using R, a one-way analysis of variance (ANOVA) was carried out for each species, to determine whether mean oxygen production differed among the temperature treatments for each species. Assumptions of homoscedasticity and normality were tested for each case. Where means were shown to be significantly different, a Tukey post-hoc test was carried out to see where differences occurred. Results were considered statistically significant at the 95 % level ($p < 0.05$).

Results

During the thermal stress experiments, thallus deterioration was present, which was not evident from the oxygen readings measured. Thallus deterioration was recorded in both the *Ecklonia maxima* and *Macrocystis pyrifera* recruits, which were exposed to temperatures in the upper end of the thermal gradient (Tab. 4). At 15 and 17.5 °C, recruits of both *E. maxima* and *M. pyrifera* displayed minimal to no thallus deterioration. At 20 °C, 80 % of the *M. pyrifera* recruits and 40 % of the *E. maxima* recruits displayed signs of thallus deterioration. This increased to 100 % of *M. pyrifera* recruits and 80 % *E. maxima* recruits in the 22.5 °C treatment, exhibiting signs of thallus deterioration (Tab. 4). Deterioration in the *E. maxima* recruits predominantly occurred along the lamina margins (Fig. 17), whereas deterioration in the *M. pyrifera* recruits was observed throughout the lamina (Fig. 18).

**Table 4.** Percentage of South African *E. maxima* and *M. pyrifera* recruits, from each temperature treatment, showing observed signs of thallus deterioration post thermal stress experiment.

| Temperature (°C) | Percentage of samples showing signs of thallus deterioration (%) | |
|---|---|---|
| | *M. pyrifera* | *E. maxima* |
| 15 (control) | 0 | 0 |
| 17.5 | 20 | 0 |
| 20 | 80 | 40 |
| 22.5 | 100 | 80 |

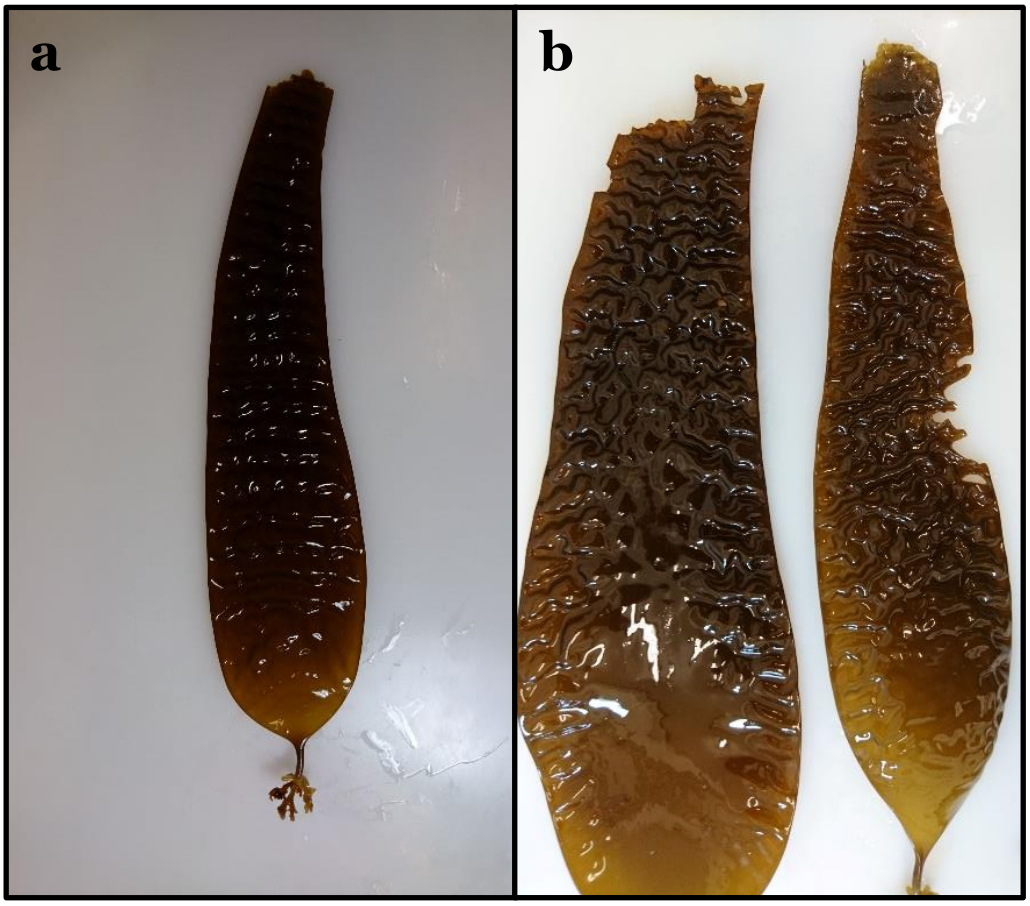


**Figure 17.** Observations during the *E. maxima* thermal stress experiment. **a** an *E. maxima* recruit prior to being subjected to the thermal stress period; **b** an *E. maxima* recruit post thermal stress period, from the 22.5 °C treatment, exhibiting signs of marginal deterioration.

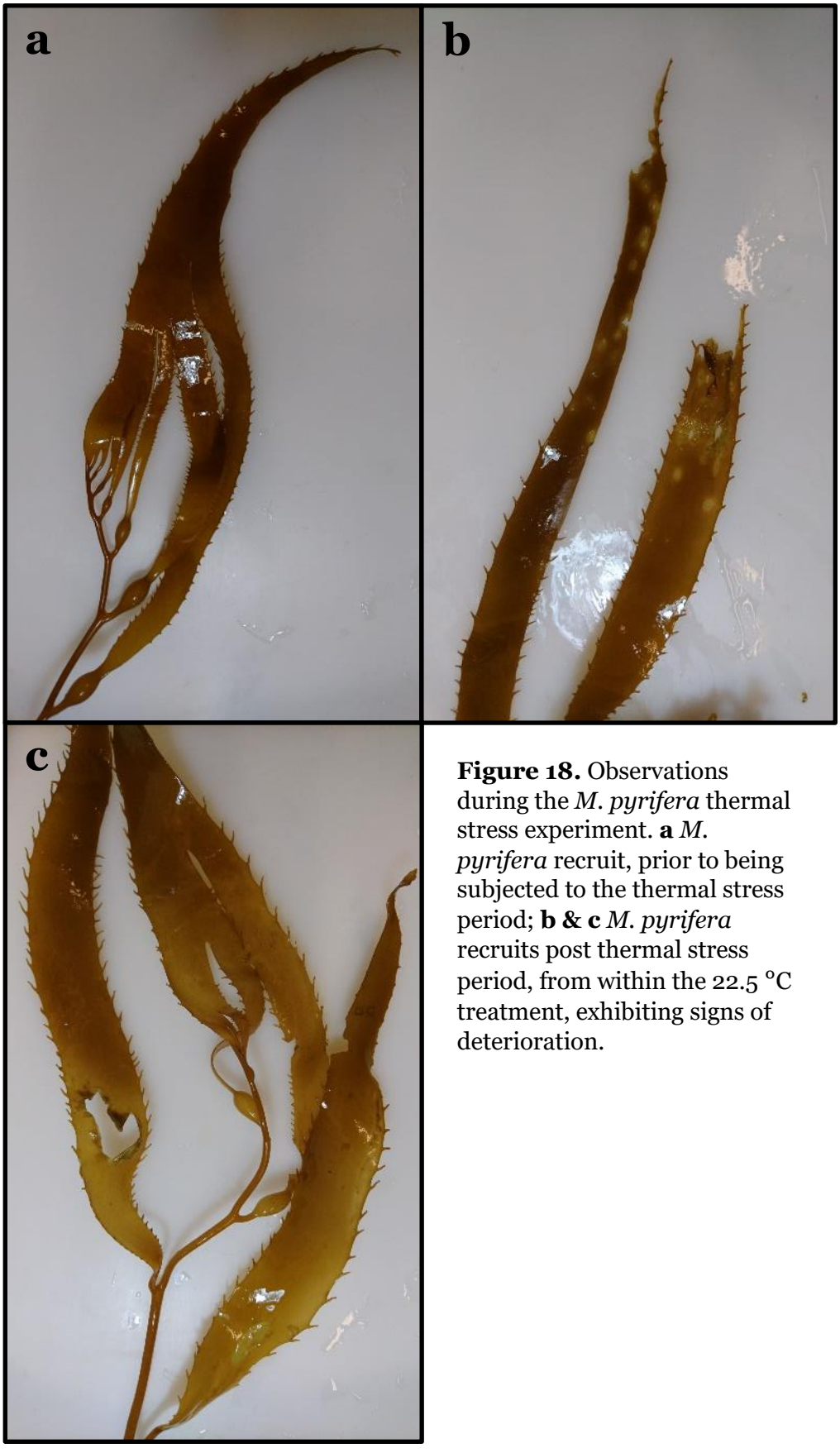


**Figure 18.** Observations during the *M. pyrifera* thermal stress experiment. **a** *M. pyrifera* recruit, prior to being subjected to the thermal stress period; **b & c** *M. pyrifera* recruits post thermal stress period, from within the 22.5 °C treatment, exhibiting signs of deterioration.

The mean rate of oxygen production by *E. maxima* recruits was found to be highest in the 17.5 °C temperature treatment, at 1.92 ± 0.19 mg $O_2.g^{-1}$ $DW.h^{-1}$ and lowest in the 22.5 °C temperature treatment, at 1.07 ± 0.09 mg $O_2.g^{-1}$ $DW.h^{-1}$ (Fig. 19). *Ecklonia maxima* recruits in the 15, 17.5 and 20 °C temperature treatments displayed similar oxygen production rates post thermal stress, with no significant differences among these means. However, the mean oxygen production rate of *E. maxima* recruits in the 22.5 °C temperature treatment was found to be significantly less than those of the other treatments ($F= 4.987$, $df_1= 3$, $df_2= 16$, $p< 0.05$) (Fig. 19).

Mean rate of oxygen production by *M. pyrifera* recruits was found to be highest in the 15 °C temperature treatment, at 1.42 ± 0.12 mg $O_2.g^{-1}$ $DW.h^{-1}$ and lowest in the 22.5 °C temperature treatment, at 0.29 ± 0.13 mg $O_2.g^{-1}$ $DW.h^{-1}$ (Fig. 19). The mean oxygen production rate declined with each subsequently higher temperature. No significant differences in the mean oxygen production rate were found between the 15 and 17.5 °C temperature treatments and between the 17.5 and 20 °C treatments. However, the oxygen production rate was found to be significantly lower in the 20 and 22.5 °C treatments, in comparison with the 15 °C treatment ($F= 18.410$, $df_1= 3$, $df_2= 16$, $p< 0.05$) (Fig. 19).

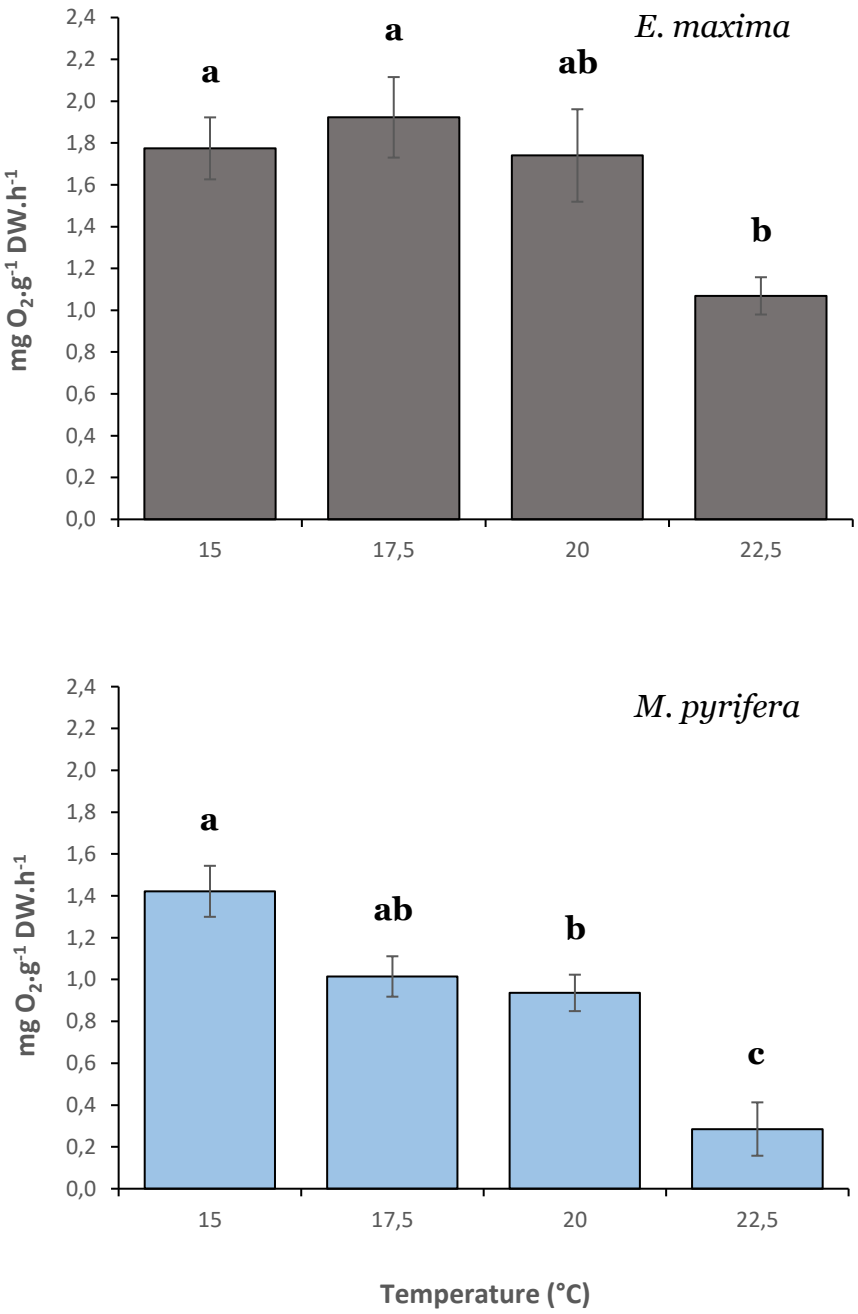


**Figure 19.** Mean oxygen production by South African *E. maxima* (top) and *M. pyrifera* (bottom) recruits, in response to exposure to a range of water temperatures for a three day period, followed by a one hour recovery period at the 15 °C control temperature. Bars represent standard error (n=5). Different letters above bars represent significantly different means among the temperature treatments for each species.

## Discussion

Rising ocean temperatures are inevitable in our future. Higher ocean temperatures threaten marine species that rely on cooler waters for normal functioning. In particular, global kelp populations and the ecosystems they support are at risk. Here I investigated the impact of Marine Heat Waves on two co-occurring South African kelp species, *Ecklonia maxima* and *Macrocystis pyrifera*.

The findings of this investigation align with my initial predictions, as they suggest that *E. maxima* and *M. pyrifera* exhibit differing thermal tolerance ranges. However, both species displayed impaired productivity and increased thallus deterioration when exposed to higher temperatures. *Macrocystis pyrifera* was found to display a greater sensitivity to higher ocean temperatures, in comparison with *E. maxima*. This is likely due to adaptation by *M. pyrifera* to higher latitude environments. Strong evidence supports the notion of *M. pyrifera* originating in the cold temperate waters of the high latitude North Atlantic (Graham et al., 2007; Schiel & Foster, 2015). Additionally, current *M. pyrifera* populations show a pattern of anti-tropical distribution occurring predominantly in cold temperate and sub-Antarctic localities (Coyer et al., 2001). Therefore, one can expect *M. pyrifera* to display a thermal tolerance range and thermal optimum in alignment with its biogeographic origins and present-day distributions, with preferred temperatures coinciding with high latitude and cold temperate conditions.

This pattern of thermal tolerance can be seen in the various global distributions of *M. pyrifera*. For example, Chilean *M. pyrifera* persists in water temperatures of 8 - 18 °C and canopy die-off is common in summer when temperatures exceed this range (Buschmann et al., 2004). Buschmann et al. (2004) also found that *M. pyrifera* in

southern Chile presented higher sporophyte growth rates at 8 and 15 °C, in comparison with individuals at 18 °C. Additionally, *M. pyrifera* in northern Chile was found to favour low temperatures (8 – 15 °C) for zoospore release, germination and recruit development. In southern California, Dean & Jacobsen (1984) linked high temperature and fouling, with reduced growth in juvenile *M. pyrifera* kelp. In their study the growth rate of juvenile samples was highest at a range of 15 – 17 °C, but was reduced at temperatures above this range (Dean & Jacobsen, 1984). Furthermore, Deysher & Dean (1986) found that temperatures above 16 °C had strong inhibitory effects on the recruitment of *M. pyrifera* in southern California, while Muth et al. (2019) recorded Californian *M. pyrifera* to begin showing recruitment failure at 18 °C. In Baja California, Mexico, Ladah et al. (1999) reported total losses of *M. pyrifera* at their southernmost limit during the 1997/98 ENSO. These populations are noted as having particularly high temperature tolerance, existing within an annual temperature range of approximately 17 – 22 °C (North, 1971; Ladah et al., 1999). Despite this, temperature anomalies during the ENSO event exceeded this range by up to 4 °C, causing local forests to disappear and only began recovering the following summer post-ENSO (Ladah et al., 1999). Around Tasmania, Johnson et al. (2011) reported gradual reductions in *M. pyrifera* populations since the 1980's, which has been linked to the intensification of the East Australian Current, resulting in an influx of warm nutrient poor water. Since the early 1950's, mean annual ocean temperatures along Tasmania's east coast have risen from approximately 13 °C to above 15 °C measured in 2010, with subsequent declines in *M. pyrifera* populations of up to 98 % recorded at some sites (Johnson et al., 2011).

Despite possible differences in the thermal tolerance ranges among global *M. pyrifera* populations (resulting from local adaptation and genetic variability), ocean

temperatures at and above 18 °C seem likely to lie at the upper end of the tolerance range for most global *M. pyrifera* populations. The findings of this chapter align with this tolerance range, where the greatest rate of oxygen production was achieved at 15 °C, with a temperature optimum potentially even lower than what was tested. Additionally, temperatures of 20 °C or higher had strong inhibitory effects on *M. pyrifera* oxygen production and resulted in increased thallus deterioration.

These findings are particularly relevant to the current distribution of *M. pyrifera* in South Africa. Annual mean temperatures along the west and south-west coastline range from 12 - 16 °C, however, summer monthly mean temperatures up to 19 and 22 °C have been recorded at sites west and east of the Cape Peninsula in the southern Benguela region, respectively (Stegenga et al. 1997). The findings of this chapter show that temperatures higher than 17.5 °C are likely to inhibit productivity and restrict the distribution of South African *M. pyrifera*. The present populations of *M. pyrifera*, therefore persist in locations/refuges where monthly mean temperatures are consistently below 17.5 °C, as well as where a combination of other environmental factors (wave action, light availability, nutrient availability, etc.) suit the preferences of this species. For example, these findings explain why no populations of *M. pyrifera* have been recorded east of Cape Point, especially in False Bay, South Africa, where temperatures often exceed 18 °C (detached *M. pyrifera* uprights have been recorded washed up in Muizenberg, False Bay, however, no attached populations have been found south or east of Kommetjie) (Bolton et al., 2012; Smit et al., 2017). Additionally, although west coast temperatures are cooler, wave action and other unfavourable conditions (lack of suitable substrate, light availability), as well as interspecific competition, may be preventing *M. pyrifera* from expanding further up the west coast- South African *M. pyrifera* exists only in sheltered localities (undulations present on

the blades of this kelp species are thought to allow for better uptake of nutrients in a low flow environment (Neushul, 1972; Wheeler, 1980; Norton et al., 1982; Hurd, 2000), which may provide a competitive advantage over *E. maxima* and *L. pallida* in sheltered locations), while the northern half of the west coast has few sheltered embayments and experiences wave heights of up to 5 m (Rothman et al., 2017).

*Ecklonia maxima* displayed a greater level of tolerance to warming temperatures, in comparison with *M. pyrifera*. Unlike *M. pyrifera*, *E. maxima* achieved increased oxygen production at a higher temperature of 17.5 °C, above which oxygen production decreased. The distribution of *E. maxima* encompasses a much greater length of the South African coast line, including False Bay and many localities east of the Cape Peninsula (Bolton et al., 2012). This distribution also spans a larger range of ocean temperatures, including temperatures reaching monthly means of 20 °C at its eastern distribution limit (Stegenga et al., 1997; Smit et al., 2017). Therefore, the thermal tolerance range of *E. maxima* is likely larger, with a thermal optimum at a higher temperature than that of *M. pyrifera*. This aligns with the findings of Bolton & Levitt (1985), who suggested that *E. maxima* was a cool temperate species and showed that gametophyte growth was greatest at 17.5 and 20 °C. Furthermore, Bolton & Anderson (1987) found juvenile *E. maxima* sporophytes (1 mm in length) to withstand a wide range of temperatures (8 - 22 °C), above which growth rates slowed. In comparison with the closely related *Ecklonia radiata* (Rothman et al., 2015), early work by Novaczek (1984) found that individuals from Goat Island Bay, New Zealand, exist in a temperature range of 10.7 – 23.9 °C. These individuals displayed a broad temperature optima of roughly 12 – 20 °C, with temperatures exceeding 24 °C resulting in kelp death (Novaczek, 1984). Variation in temperature tolerance likely exists within this genus, among global populations and at the differing life stages of this species.

Interestingly, *E. maxima* displayed a greater tolerance to higher ocean temperatures than *M. pyrifera*, despite being sampled from an identical habitat and was exposed to identical pre-experiment acclimatisation, emphasising its cool temperate nature. Although *E. maxima* may be more tolerant to higher ocean temperatures, this species still displayed reduced oxygen production and increased thallus deterioration at 22.5 °C. This aligns with the temperature regime along the current *E. maxima* distribution, where monthly mean temperatures are unlikely to exceed 22.5 °C (Bolton & Levitt, 1985).

Despite differing thermal tolerances, both species proved susceptible to prolonged exposure to temperatures above 20 °C, especially at the 22.5 °C level. Predicted increases in Marine Heat Wave frequency and intensity will likely negatively impact *E. maxima* and *M. pyrifera* populations in the future, particularly as MHW's around South Africa last on average ± 9 days (Schlegel et al., 2017), whereas samples in this study were subjected to only three days (72 hours) of elevated temperatures. This has serious implications for those kelp populations that exist in locations where temperatures already extend into the upper limit of temperature tolerance of each species (Wernberg et al., 2017) (i.e. False Bay and the east of the Cape Peninsula, where sea temperatures are warmer (Smit et al., 2017)). Reductions in the abundance of *E. maxima* will have consequences for those industries that rely on its commercial availability. This includes the commercial plant-growth stimulant industry (Kelpak® and Afrikelp®) and abalone farms that use fresh kelp as abalone feed (Anderson et al., 2003; Troell et al., 2006; Amosu et al., 2013; DAFF, 2016b), particularly as the greatest number of kelp-using farms occur east of the Cape Peninsula where *E. maxima* could be most impacted by warming (DAFF, 2016a). Moreover, reductions in both *E. maxima* and *M. pyrifera* will have negative implications for associated kelp forest

organisms, the ecosystems they sustain and the ecosystem services these kelp forests provide. For example, *E. maxima* forests support diverse ecosystems and provide habitat to three obligate, red algal epiphytes (Anderson et al., 2006). Additionally, while little is known about the organisms associated with South African *M. pyrifera*, it has been found to harbour a unique array of holdfast biota (Fleischman, 2016).

Lastly, many South African studies have highlighted an ecosystem regime shift characterised by a cooling of south-west coast average ocean temperatures, which has allowed for an eastward expansion in the range of *E. maxima*, as well as an increase in the abundance of this species within its present distribution (Blamey et al., 2012, 2015; Bolton et al., 2012; Reimers et al., 2014). However, an increased frequency and intensity of future warm events such as MHW's, along the west and south-west coast, may offset regime shifts such as these and hinder the expansion of cool temperate species along the South African coastline.

In conclusion, both *M. pyrifera* and *E. maxima* displayed reduced oxygen production and increased thallus deterioration when subjected to increased water temperatures for a 72 hour period. Moreover, *M. pyrifera* displayed a lower temperature threshold and greater vulnerability to rising ocean temperatures, in comparison with *E. maxima*. An increasing frequency and intensity of MHW's and future predictions of rising ocean temperatures could negatively impact the *E. maxima* and *M. pyrifera* populations of South Africa, with an impact on the former potentially resulting in dire associated social, economic and ecological consequences.

Chapter 4-

General discussion

In South Africa, populations of *Macrocystis pyrifera* exist only in the extreme south of the west coast. Little information is available on these kelp populations, as little research has been conducted on them. South African *M. pyrifera* is found in only eight locations, predominantly within enclosed bays, and often bordered offshore by *Ecklonia maxima* kelp forests (Papenfuss, 1942; Stegenga et al., 1997; Anderson et al., 2007, pers. obs.) (Fig. 7). These *M. pyrifera* populations form mounding rhizomatous holdfasts, similar to those found along the southern Australian coast (Graham et al., 2007). With the exception of the Dassen Island and Robben Island populations, South African populations exist only at depths of 1 – 2 m at neap tide, on rocky substratum, with fronds extending to the surface and becoming exposed during low tides. On Dassen Island and Robben Island the populations exist on the eastern side of each island, where there is shelter from the prevailing south-westerly swells (Mark Rothman, Department of Environment, Forestry and Fisheries, pers. comm.). The island populations are observed to grow longer and can be found at greater depths than the mainland populations, with *M. pyrifera* uprights at Robben Island recorded at 6 m length and holdfasts at a depth of 6.3 m (Derek Kemp, Department of Environment, Forestry and Fisheries, pers. comm.). An assessment of morphological characteristics carried out by Fleischman (2016), found the uprights of South African *M. pyrifera* to occur in high densities, up to 160 uprights per 0.25 $m^{-2}$, and have highly interwoven holdfasts, inhibiting distinction between individuals. The uprights of the mainland populations don't exceed more than 3 m in length and are shorter in comparison with most other global populations. Additionally, similar to the work carried out by Westermeier et al. (2013, 2016) on Chilean *M. pyrifera*, Fleischman

(2016) found South African *M. pyrifera* has the potential to be cultivated through vegetative propagation on rope rafts, displaying upright elongation rates of 0.99 – 1.22 %.day$^{-1}$ over a three month winter/spring growing period.

This thesis aimed to increase our knowledge of South African *M. pyrifera* and focussed on investigating the use of *M. pyrifera* as an alternative source of abalone feed and assessing the impact of Marine Heat Waves (MHW's) on *M. pyrifera* and the ecologically dominant *Ecklonia maxima*. *Macrocystis pyrifera* was determined to be a suitable alternative source of supplementary kelp feed for the abalone aquaculture industry, providing similar growth rates to abalone fed with *E. maxima*, which is widely used as feed on local commercial abalone farms. Furthermore, the findings of the simulated MHW experiments highlighted the sensitivity of *M. pyrifera* and *E. maxima* to rapid increases in ocean temperatures and provided insight into the temperature-related physiological constraints of both species within their present distributions.

<u>The feasibility of *Macrocystis pyrifera* cultivation and use in South Africa</u>

*Macrocystis pyrifera* is an important feed species within the Chilean abalone aquaculture industry (Flores-Aquilar et al., 2007; Correa et al., 2016). Abalone farming in Chile has increased from 73 t in 2001 to 840 t in 2011, quickly raising demands for fresh kelp feed (Buschmann et al., 2014). As a result, total landings from natural *M. pyrifera* populations in Chile rose from 9,672 to 19,400 t between 2001 and 2011 (Statistical yearbook, SERNAPESCA, 2011). Consequently, the exploitation of natural *M. pyrifera*, particularly in central-northern Chile, has reached maximum sustainable levels (Buschmann et al., 2014). Limitations on macroalgal biomass and a rapidly growing abalone industry has fuelled research into the sustainable cultivation

of *M. pyrifera* in Chile. The majority of pilot projects focussed on cultivating free-floating or rope seeded *M. pyrifera* on suspended rope raft systems (see Westermeier et al., 2006, 2011, 2013, 2016; Macchiavello et al., 2010; Buschmann et al., 2014; Camus et al., 2018; Correa et al., 2016; Camus & Buschmann, 2017a, b). The most recent and largest of these projects, a three year production cycle carried out over 21 ha in southern Chile, was able to produce 124 wet $t.ha^{-1}.month^{-1}$ and highlighted the feasibility of *M. pyrifera* commercial cultivation (Camus et al., 2018). This industry is in its infancy (commercial operations have not begun), having only cultivated 2 t in 2014, however, continued efforts may see beneficial outcomes that support future growth in the Chilean abalone industry (Buschmann et al., 2014; Camus & Buschmann, 2017a).

Unlike Chile, the South African abalone aquaculture industry is not fully dependent on macroalgal biomass for abalone feed, due to the inclusion of formulated feeds on all abalone farms (Troell et al., 2006; Bolton et al., 2009). Additionally, farms along the west and south-west coast that depend on a supply of fresh kelp, are not restricted by its natural availability, with this resource being well managed along the coast (DAFF, 2016b). Nonetheless, almost all of South Africa's abalone farms view fresh seaweed as an important addition to formulated feed as abalone diet, either using cultivated *Ulva spp.* or harvested kelp (or a combination of both) (Naidoo et al., 2006; Troell et al., 2006; Bolton et al., 2013; DAFF, 2016b). For example, *ca.* 2,000 t fresh *Ulva* is cultivated in recirculating raceways on South African abalone farms per annum and just over 4,000 t of fresh kelp is harvested annually for abalone feed (Bolton et al., 2016; DAFF, 2016b; Mark Rothman, Department of Environment, Forestry and Fisheries, pers. comm). Moreover, improving and optimizing abalone diets to better production efficiency is a key priority in the industry (see Sales & Britz, 2001; Naidoo

et al., 2006; Troell et al., 2006; Dlaza et al., 2008; Francis et al., 2008a,b; Nel et al., 2017a,b). Therefore, research into the improvement of abalone feeds and the determination of abalone feed alternatives is a worthwhile objective, which may be accompanied by cost and production benefits.

Much can be discussed about the feasibility of *M. pyrifera* cultivation and its use in the South African abalone industry. The findings of Chapter 2 suggest that *M. pyrifera* is a suitable supplementary kelp feed for *Haliotis midae*, providing comparable growth results to the widely used *E. maxima*. Additionally, the reduced consumption rates seen by abalone fed with *M. pyrifera*, in comparison with *E.* maxima, hint at potential differences in the nutritional make-up of *M. pyrifera*. For example, *M. pyrifera* may provide production orientated benefits such as a reduced Feed Conversion Ratio or a differential availability of nutrients to the abalone, although further investigation is needed to confirm these notions. Furthermore, despite these findings, the small populations and low biomass of *M. pyrifera* in South Africa, means that natural availability is limited and would need to be commercially cultivated to meet future potential sustainable demands.

Although Fleischman (2016) presented a feasible way of sustainably cultivating *M. pyrifera* through vegetative propagation, doing so was not without its difficulties. Any form of mariculture requires a set of specific environmental conditions, which allow for the efficient farming of the organism (Duarte et al., 2017). Much of the world's aquaculture takes place in enclosed bays or fjords, where water motion is still present (an essential requirement for algal nutrient and gas uptake (Hurd, 2000)), but wave action is reduced enough to not damage the infrastructure or organisms (see Forbord et al., 2012; Broch et al., 2013; Handå et al., 2013; Marinho et al., 2015; Stévant et al.,

2017). The South African coastline experiences high levels of wave action, up to 5 – 7 m maximum swell height along the west and south-west coast (Rothman et al., 2017), making offshore mariculture problematic.

At present, most of South Africa's offshore mariculture takes place in its few large embayments and estuaries and primarily comprises of bivalve mariculture of the exotic oyster species, *Crassostrea gigas* 'Pacific oyster', and two mussel species, the exotic Mediterranean mussel, *Mytilus galloprovincialis* and the indigenous black mussel, *Choromytilus meridionalis* (DAFF, 2016a). Saldanha Bay, located on the west coast of South Africa, represents the most mariculture-active location, with four oyster and four mussel farms operating as of 2015. Other farms on the west coast also include two oyster farms, one in Paternoster and the other in Kleinzee. On the south coast, one oyster farm exists in Knysna Lagoon and on the east coast, a further two oyster farms are located in Port Elizabeth and one in Jeffrey's Bay (DAFF, 2016a).

The *M. pyrifera* pilot cultivation project, carried out by Fleischman (2016), took place in the inner bay of Saldanha Bay. Saldanha Bay forms part of the Langebaan lagoon system and is the largest deep-water bay in the country (Anderson et al., 1996a). The bay is split into an inner and outer bay, divided by an ore jetty constructed in 1974. The inner Saldanha Bay experiences monthly mean swell heights below 2 m and is thus considered a sheltered bay with low wave action (Anderson et al., 1993). Apart from bivalve mariculture, many other activities take place within Saldanha bay including fishing, oil and ore loading and various recreational and tourism activities (Anderson et al., 1996a, 1999).

Saldanha Bay has also been the site of historic activities, including fish processing and *Gracilaria* (*G. gracilis* (Stackhouse) Steentoft, L.M.Irvine & Farnham 1995: 115) beach cast collection. However, the fish processing factories have since closed (Anderson et al., 1999; Stenton-Dozey et al., 1999), and the construction of the ore jetty and breakwater in the 1970's caused the collapse of the *G. gracilis* resource in the late 1980's, ending commercial collections (Anderson et al., 1993; Rothman et al., 2009). The mariculture of bivalves (initially only mussels) on raft systems within Saldanha Bay began in 1984 (Stenton-Dozey et al., 1999). In 2015, the mussel sub-sector, which currently solely occurs in Saldanha Bay, produced 1,759 t of product. This comprises 49 % of the total South African aquaculture production, making it the largest contributor to aquaculture production by weight in the country (DAFF, 2016a). Additionally, oyster farms on the west coast contributed to 89 % of total oyster production, with four of the six west coast farms occurring in Saldanha Bay (DAFF, 2016a).

In addition to present aquaculture activities, in 2018 the Department of Environment, Forestry and Fisheries (DEFF) acquired approval for the implementation the Saldanha Bay Aquaculture Development Zone (ADZ). In line with meeting Operation Phakisa: Ocean Economy (a state run project aimed at unlocking the economic potential of South Africa's oceans) production targets, the ADZ aims to increase the available area within Saldanha Bay for aquaculture activities, increasing job creation, primary production and promoting growth in South Africa's aquaculture sector (DAFF, 2018). The proposed new locations will allow for aquaculture using fish cages, long lines and rafts, with the aim of producing mussels, oysters, fin fish, abalone, scallops and seaweeds. At present, 464 ha in Saldanha Bay are allocated for aquaculture, with 151 ha currently used. After implementation of the ADZ, this number will increase to 884

ha available for various forms of aquaculture (DAFF, 2018). Therefore, Saldanha Bay is a location characterised by high levels of industrial and commercial activities, with a large potential for future aquaculture related investigations to be carried out.

Saldanha Bay experiences a pattern of strong seasonal differences in nutrient levels and water temperature within the water column. During summer, surface warming due to increased insolation and a movement of cold nutrient rich waters into the bay, creates a warm oligotrophic surface layer divided by a thermocline (Anderson et al., 1996b). The thermal stratification and separation of the surface layer begins to form in spring and by summer this layer becomes eutrophic through phytoplankton activity (Anderson et al., 1996b, Anderson et al., 1999). The thermocline ranges in depth from 5 to 10 m and tends to shift in depth over a 6 - 7 day cycle. During this cycle, cold nutrient rich waters may reach the surface waters through advection (Anderson et al., 1999). In winter, stratification does not occur within the bay, as prevailing north-westerly winds cause strong internal mixing. This results in a well-mixed water column with fairly constant water temperatures and nutrient availability (Anderson et al., 1996b). In outer bay, Saldanha Bay, monthly mean SST's range from 14 to 16 °C in autumn/winter and 16 to 20 °C in spring/summer (Monteiro & Brundrit, 1990). In inner bay, daily mean SST's range from 10 to 14 °C in winter and 17 to 20 °C in summer, with temperatures above 17 °C indicating oligotrophic conditions (Anderson et al., 1996b).

During the *M. pyrifera* cultivation pilot cultivation project (Fleischman, 2016), although wave action was not an issue in the inner bay, the hydrological characteristics of the chosen location produced potentially undesirable conditions for kelp cultivation. The warm nutrient poor surface layer that forms in early spring, a high

prevalence of fouling organisms (which occur in large densities around the oyster/mussel lines) and a lack of water motion were likely contributors to a high degree of fouling and poor quality biomass seen in the samples (despite the high elongation growth rates recorded). In early 2017, attempts were remade to cultivate *M. pyrifera* in the outer bay of Saldanha Bay, which is closer to the lagoon mouth. It was hoped that the increased water motion would provide more suitable conditions for cultivation, however, a series of strong storms repetitively damaged the experimental setup, after which further attempts to continue with the project were ceased.

Despite these challenges, Saldanha Bay is one of the few locations where this form of kelp mariculture could take place, due to its enclosed environment, pre-existing aquaculture infrastructure and a large potential for future aquaculture expansion. The future of *M. pyrifera* cultivation is thus reliant on finding a suitable location (either in Saldanha Bay or elsewhere) where substantial water movement is present, but is protected from high levels of wave action. Moreover, the findings from Chapter 3 stress the need for the chosen location to provide consistently low water temperatures (< 17.5 °C), allowing the cultivated kelp to remain productive. Given these conditions, it is suggested that prior to any further attempts to cultivate this species within Saldanha Bay and particularly inner bay, suitable sites for cultivation, which provide the necessary conditions for culture, need to first be investigated. Additionally, suitable infrastructure needs to be investigated for long term cultivation, as well as alternative methods to propagate *M. pyrifera*, i.e. spore culture (see Gutiérrez et al. 2006, Westermeier et al. 2006, 2011, Macchiavello et al. 2010, Correa et al. 2016; Camus & Buschmann, 2017b).

Further pilot scale trials to optimise *M. pyrifera* cultivation are therefore required, as has been essential in all of South Africa's various aquaculture sectors. Moreover, carrying out further ecological studies on *M. pyrifera* will increase our knowledge about the physical and ecological requirements of this species, aiding in its successful utilisation. Nonetheless, I remain optimistic that through scientific experimentation, a method of cultivating this species for long term sustainability will be made possible.

Abalone aquaculture in South Africa has been increasing for many years and farmers are continually searching for new sustainable sources of abalone feed. Despite current kelp harvesting levels not exceeding MSY, farms that continue to use kelp as a feed will likely require increased amounts of fresh kelp biomass in the future as more farms are built or existing farms expand, and will thus benefit from the cultivation and use of fresh *M. pyrifera* as an alternative abalone feed. Furthermore, cultivated *M. pyrifera* could also be included within formulated feeds, meaning that *M. pyrifera* may have uses other than as fresh kelp feed. For example, the South African company Taurus Products (Pty) Ltd. developed a macroalgal based formulated abalone feed called 'Midae Meal', which predominantly comprised of *Laminaria pallida* and *E. maxima* (Troell et al., 2006). Moreover, the Mexico Based Company AlgaMar™ currently manufactures a kelp based formulated feed product called ABKelp®, which primarily comprises of *M. pyrifera*. In addition to the possible benefits to abalone farms, sustainably growing kelp could reduce the level of harvesting occurring on natural South African kelp forests, which will aid in reducing the impacts of harvesting on biodiversity (see Christie et al., 1998; Anderson et al., 2006).

Lastly, since the 19$^{th}$ century, the ocean has absorbed 50% of $CO_2$ produced and retained twenty times more heat than the atmosphere. Consequences of this include a

lowering of ocean pH, resulting in ocean acidification, and a steady rise in sea surface temperatures (SST's) (Sabine et al., 2004; Levitus et al., 2005). By 2150, atmospheric $CO_2$ is predicted to triple, ocean pH decrease by 0.3 units and increases in SST's of 1.4 – 5.8 °C (IPCC, 2007; Shukla & Edwards, 2017). Promoting macroalgal cultivation will see a wealth of climate change mitigation related benefits to combat these changes. As was highlighted by Duarte et al. (2017), macroalgal farming could play a pivotal role in combating climate change, through carbon sequestration, biofuel production, dampening wave energy to protect shorelines, serving as a safer alternative to synthetic fertilisers, increasing water column oxygen levels and elevating ocean pH (reducing the impacts of de-oxygenation and ocean acidification). Moreover, nutrient recycling carried out by cultivated *M. pyrifera* could aid in alleviating nutrient loading in industry-heavy locations and promote bioremediation (Buschmann et al., 2008; Teagle et al., 2017).

MHW's and South African *M. pyrifera* and *E. maxima* populations

It is difficult to predict the impact of an increasing frequency and intensity of ocean warming events such as MHW's, on South Africa's *E. maxima* and *M. pyrifera* populations. Despite this thesis highlighting the sensitivity of both species to MHW's, the hydrological characteristics of the South African coastline are unique and complex. This makes predicting how *E. maxima* and *M. pyrifera* will respond to ocean warming events a difficult task. For example, although the southern Benguela has been identified as an ocean warming hotspot (Hobday & Pecl, 2014), findings by Rouault et al. (2010) showed that coastal seas along the west and south-west coastline are instead cooling at a rate of 0.5 °C per decade. This trend of cooling was suggested to be the result of upwelling intensification driven by shifts in decadal wind regimes (an increase in south-easterly winds since the 1990's), which are itself influenced by

climate change (Rouault et al., 2010; Blamey et al., 2012, 2015). This has been accompanied by range expansions for many coastal marine organisms, including an eastward range expansion of *E. maxima*, as well as the expansion of this species into previously uninhabited locations such as the inner regions of False Bay (Griffiths & Mead, 2011; Bolton et al., 2012; Reimers et al., 2014; Blamey at al., 2015). Additionally, Bolton et al. (2012) suggested that if coastal cooling continues we can expect to see further eastward expansions in *E. maxima* and other kelp forest distributions. Therefore, areas where cooling is most prominent may serve as refuges for South African kelp populations during MWH and other anomalous warming events.

It is also necessary to distinguish between the threat of gradual ocean warming and MHW's when investigating their impacts. Gradual ocean warming may create an environment that inhibits the long term recovery of temperate kelp forests (see Johnson et al., 2011), while MHW's only persist for a short time, after which conditions may return to normal. As a result, we do not yet know the long term implications of the occurrence of MHW's along the South African coastline or whether kelp populations will recover post-MHW. For example, ENSO events in southern California and Baja California, Mexico, have often resulted in the disappearance of local *M. pyrifera* populations, with monthly mean SST's reaching 25 °C at peak event intensity (Tegner & Dayton, 1991; Tegner et al., 1996; Ladah et al., 1999). However, these populations are known to quickly recover within a few months (Tegner & Dayton, 1991; Ladah et al., 1999). Additionally, the warming events that caused large scale reductions (±100 km range contraction) in *Ecklonia radiata* populations along the west coast of Australia (Wernberg et al., 2013, 2016) have only occurred relatively recently and whether these kelp forests will eventually recover is unknown.

Finally, taking into account the potential differential impacts of MHW's on the differing life stages of kelp species is also necessary to understanding how species will respond. For example, Bolton & Levitt (1985) showed that female *E. maxima* gametophytes are able to survive in a temperature range of 3 – 25 °C and tom Dieck (1993) found Californian *M. pyrifera* gametophytes to persist in a temperature range of -1.5 - 26 °C. In comparison with the findings in Chapter 3, the gametophytes of both species are able to persist in a far larger range of ocean temperatures than sporophytes of the same species. Therefore, it will likely be the sporophytes of each species that will be more prone to MHW induced stresses.

Consequently, in coastal locations where temperatures already present relatively high monthly means (Fig. 20), MHW's could result in the degradation of local *E. maxima* and *M. pyrifera* forests. Furthermore, MHW's will likely have a varying impact on each species, due to differences in physiology and distribution. For instance, where these kelp species co-occur, *M. pyrifera* may be more prone to stress and degradation by MHW's than *E. maxima*, as South African *M. pyrifera* was shown to produce significantly reduced oxygen and increased upright deterioration at 17.5 °C, while *E. maxima* was able to better withstand temperature up to 20 °C.

Alternatively, *M. pyrifera* could display improved recovery to MHW's than *E. maxima*. For example, during periods of high ocean temperatures (either due to summer warming or ENSO events), *M. pyrifera* populations along the west coast of North and South America commonly show canopy deterioration or see loss of individuals under extreme warming. However, these populations begin to show signs of recovery within several months (North, 1971; Tegner & Dayton, 1991; Tegner et al., 1996; Ladah et al., 1999; Buschmann et al., 2004). Therefore, the occurrence of

MHW's in South African *M. pyrifera* could either see the loss of entire populations or simply the loss of canopies, as uprights deteriorate. If a population is lost, the sparse distribution of the South African *M. pyrifera* may make recruitment post-MHW difficult. However, if only canopy loss occurs, it is likely that South African *M. pyrifera* has the potential to regrow canopies from holdfasts (should meristematic tissue still exist) and recover post-MHW. On the other hand, *E. maxima* is unable to lose and regrow a stipe if lost (the meristematic region being located high up on the thallus between the stipe and primary blade, and between the primary and secondary blades) and an individual will likely die if thermal tolerance levels are exceeded, with recruits taking up to 3 years to reach maturity and replace lost individuals (Anderson et al., 2006; Rothman et al., 2006). As a result, *E. maxima* will likely show a reduced ability to recover post-MHW's in comparison with *M. pyrifera*. Despite this, *E. maxima* was shown to be more resilient to rising temperatures and will thus only be threatened by MHW's that cause ocean temperatures to exceed 20 °C for prolonged periods of time. Therefore, *E. maxima* populations along the cooler west coast may be able to better withstand MHW's, while those east of the Cape Peninsula may be more vulnerable to warming events, due to ocean temperatures rising eastwards (Fig. 20).

Moreover, apart from the direct implications of MHW's on kelp functioning, other biotic and abiotic factors may influence kelp forests differently in the presence of MHW's. For example, the level of impact of storm and wave activity on kelp forests may be heightened under MHW conditions. Waves and storm activity are known to serve as strong turnover agents among global kelp distributions (Santelices & Ojeda, 1984; Ebeling et al., 1985). However, rapidly warming waters will cause physiological stress among kelp and result in kelp forests being more susceptible to damage and dislodgment by high wave action (Simonson et al., 2015; Filbee-Dexter et al., 2016).

The uprights of *M. pyrifera* are known to be susceptible to high levels of wave action, with canopies regularly drastically reduced by storms along southern and central California (Ebeling et al., 1985; Seymour et al., 1989; Reed et al., 2011).

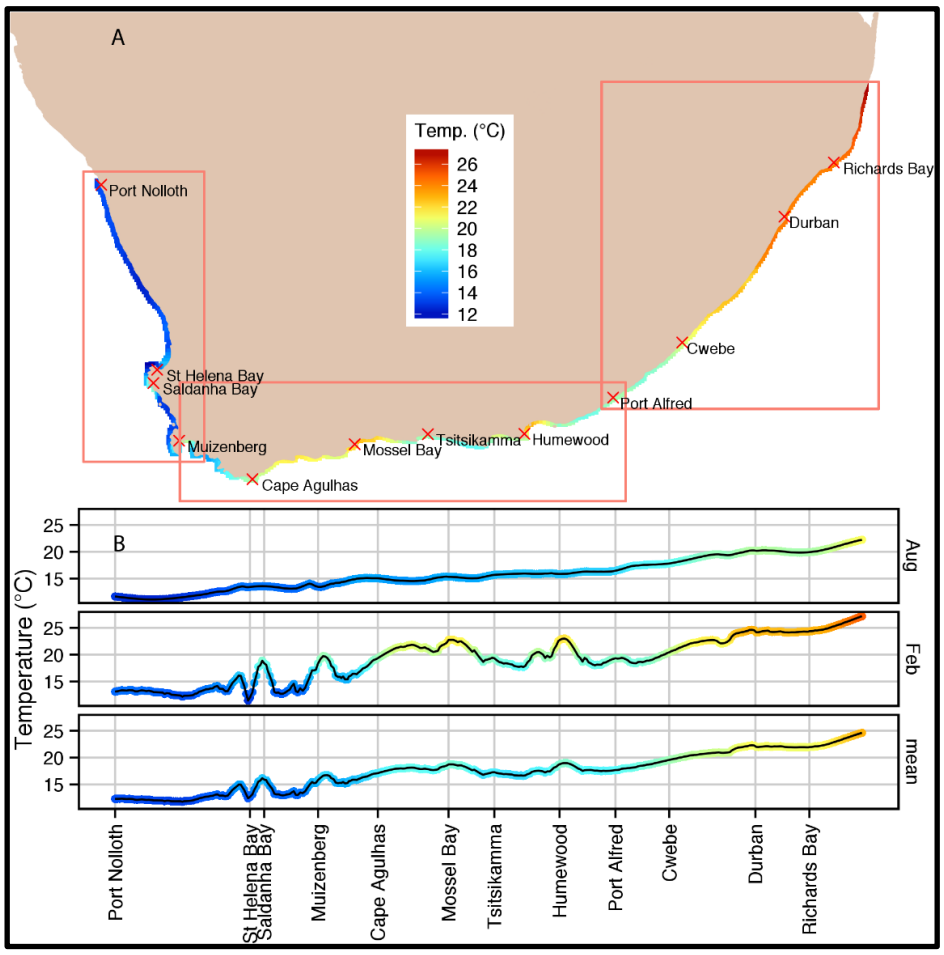


**Figure 20.** Summer inshore *in situ* temperature data for the South African coastline, showing the differences in the temperature regimes of the west, south-west and south coasts (Smit et al., 2013).

Additionally, it is well established that a strong negative correlation exists between ocean water temperature and nutrient availability (particularly nitrate, $NO_3$) (Waldron & Probyn, 1992). The relationship between South African MHW's and ambient nutrient availability has yet to be investigated, however, if nutrient availability is reduced during MHW events, increased stress could be placed on local kelp populations through nutrient limitation. This is supported by Probyn & McQuaid

(1985) who demonstrated that nitrate uptake by *E.* maxima is linearly related to ambient ocean nitrate concentration, meaning that *E. maxima* nitrate uptake would be impacted by nutrient poor conditions. Additionally, nitrate availability is often suggested to be a strong limiting factor to *M. pyrifera* productivity and growth, resulting in upright die-off when ambient nutrient levels are depleted, and directly impacts *M. pyrifera* recruitment and survival in Baja California, Mexico (Jackson, 1977; Wheeler & North, 1981; Gerard, 1982; Zimmerman & Kremer, 1986; Van Tussenbroek, 1989; Hernández-Carmona et al., 2001). Nonetheless, some kelp have the capacity to use internally stored nitrogen. Gerard (1982) and Van Tussenbroek (1989) showed that *M. pyrifera* is able to use stored nitrogen for a prolonged period of time, meaning that South African *M. pyrifera* may be able to persist through low nutrient environments potentially created by MHW's. *Ecklonia maxima* has been shown to store small amounts of nitrogen (Probyn & McQuaid, 1985; Smith, 2007), however, the length of time that this species can persist in a nutrient limited environment has not been investigated. Nonetheless, the physiological stress created by MHW's and the prevalence of high wave action along the coast of South Africa could serve as a limitation to the expansion of South African *M. pyrifera* along the west coast and the eastward movement of *E. maxima* on the south coast, as well as reduce the resilience of current kelp forest distributions.

Lastly, MHW's may also produce indirect consequences to South African kelp forests through the alteration of marine food webs and trophic cascades (Wernberg et al., 2010; Johnson et al., 2011). For example, while urchin feeding fronts do not occur in South Africa (the dominant urchin species in South Africa cannot feed on adult kelp) (Morris & Blamey, 2018), MHW driven changes to environmental conditions, macroalgal availability and key trophic species could result in alterations to herbivore

behaviour and allow for an influx of pathogens and alien fauna and flora (Krumhansl & Scheibling, 2011; Wernberg et al., 2011, 2013, 2016; Wahl et al., 2015; Filbee-Dexter et al., 2016). Additionally, the aforementioned threats may serve as future challenges to the development of a *M. pyrifera* mariculture industry. Therefore, future plans to cultivate this species will need to take both present and potential future oceanographic conditions into account.

To further understand the potential impacts of MHW's, further research is required. Studies in which kelp are subjected to longer periods of thermal stress, examine the impact of a broader range of temperatures on kelp, carry out *in situ* experiments and measure other parameters such as kelp growth rates, will allow for a more in-depth look at the effects of rising ocean temperatures and MHW's on *E. maxima* and *M. pyrifera*. This will allow for a greater level of accuracy in assessing their vulnerability to these events and enable us to better predict the future of these kelp forest ecosystems.

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